REPRODUCTIVE ECOLOGY AND MORPHOMETRIC SUBSPECIES COMPARISONS OF DUNLIN (*CALIDRIS ALPINA*), AN ARCTIC SHOREBIRD

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A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

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Fairbanks, Alaska

December 2011

ABSTRACT

The Arctic region provides globally important breeding and migratory habitat for abundant wildlife populations including migratory shorebirds. Due to their remote breeding locations, basic information on breeding ecology, annual productivity, and factors that regulate their populations are poorly studied. Wildlife biologists managing migratory bird populations require detailed information on avian breeding biology, in addition to information on migration ecology including connectivity of migratory stop-over and wintering locations. To address information gaps in fecundity, I conducted an experimental study investigating the renesting ecology of Dunlin (*Calidris alpina arcticola*) by removing clutches at two stages of incubation and by following adults marked with radio transmitters to their replacement clutch. In contrast to predictions for Arctic-breeding species, Dunlin had high (82-95%) rates of clutch replacement during early incubation and moderate (35-50%) rates during late incubation. Female body condition and date of clutch loss were important variables explaining propensity for females to replace a clutch; larger females that lost their nest early in the season were more likely to renest than smaller females who lost their nest later in the season. To delineate Dunlin subspecies in areas where they overlap, I used morphological and molecular approaches to determine sex and subspecies of five subspecies of Dunlin breeding in Alaska and eastern Russia. This analysis yielded discriminant function models to correctly classify unknown individuals to sex (79-98%) and subspecies (73-85%) via morphometric measures. Correct classification of mixed assemblages of subspecies improved when sex, determined though molecular techniques, was known. The equations I derived using discriminant function models can be used to identify the sex and subspecies of unknown Dunlin individuals for studies investigating breeding and migration ecology.

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ACKNOWLEDGEMENTS

I would like to thank each member of my graduate committee, who have contributed their knowledge, expertise and humor throughout my tenure in graduate school. My major advisor, Abby Powell offered keen and timely advice and effective constructive criticism. I thank Rick Lanctot for sharing his passion for the science and conservation of migratory shorebirds by inviting me to work with Arctic-breeding shorebirds in Barrow. Rick's unending enthusiasm, engaging discussions and dynamic support were vital to the success of my thesis. I thank Christine Hunter for her advice on data analyses and model building.

Ecological research by nature is a collaborative process and I have benefitted immensely from the feedback of fellow graduate school students including Audrey Taylor, Brooke Hill, Teri Wild, Valerie Steen, Dan Rizzolo and Roy Churchwell. Drs. Stephen Yezerinac, Liliana Naves, Brett Sandercock, Joel Schmutz, and Kevin Winker offered keen insights and ideas. Jim Johnson prepared the Dunlin subspecies range map with the assistance of Pavel Tomkovich.

I thank my field technicians for their valiant efforts to collect high quality data for long hours often times under challenging field conditions: Joaquin Aldabe, Noah Burrell, Chung Yu Chiang, Jimmy Choi, Jen Coffey, Charles Governali, Ben Haase, Jean-Francois Lamarre, Bert Lewis, Pete Mickelson, Robert Rankin. I thank my parents, John and Carol Gates, for always supporting my education.

I thank my husband, Bert Lewis, who has encouraged and supported my pursuit of avian ecology. Bert continues to foster a passionate sense of curiosity in our lives.

Lastly, I thank the Arctic and all the amazing creatures and features that embody this magnificent region. In the company of Arctic - breeding Dunlin, I have learned resilience and found inspiration in their capacity to respond and adapt to their ecological demands for survival.

CHAPTER 1: INTRODUCTION

The Arctic region is composed of millions of hectares of highly productive tundra and wetland ecosystems. These ecosystems provide critical breeding and migratory habitat for diverse and abundant wildlife populations (Callaghan 2005). Shorebirds (suborder Charadrii) are an abundant and prominent component of the Arctic avian breeding and migration communities. Due to the Arctic's remoteness, basic information on their breeding ecology, annual productivity, and factors that regulate their populations (e.g. predator-prey interactions) are poorly studied. A multitude of shorebird species travel to the Arctic to reproduce and capitalize on its relative lack of predator abundance, lower parasite pressures, more abundant food resources, and increased photoperiod for foraging (Schekkerman et al. 2003, Newton 2008, McKinnon et al. 2010). In addition, species that breed in the Arctic are exclusively long distance migrants, and have limited temporal and reproductive flexibility (Alerstam et al. 2003). Because severe seasonal and environmental conditions compress breeding and post-breeding periods for Arctic-breeding birds, it is assumed that renesting rates are reduced (Martin and Wiebe 2004).

Clutch replacement laying (e.g. renesting) is one reproductive strategy employed by birds to compensate for clutch loss and improve the odds of successfully producing offspring within the same year (Gill 2007). Causes of clutch loss include egg depredation by predators, trampling, depredation of an incubating adult, direct disturbance of clutch, and persistent unfavorable weather conditions. It is assumed that renesting propensity declines across a latitudinal gradient with species that breed at higher latitudes renesting less frequently than their temperate counterparts due to a reduction in the length of the breeding season and extreme weather conditions (Martin and Wiebe 2004). Studying renesting ecology under natural conditions proves difficult and requires that individuals are color-marked before clutch loss and that the subsequent replacement clutch is found. Discovery of replacement clutches are further complicated by divorce and movement of individuals beyond the study area. Thus, an examination of renesting ecology requires experimental removal of clutches and intensive tracking of individuals. Understanding

the factors affecting clutch replacement propensity sheds light on the importance of initiation timing and success, the effects of predation on productivity and divorce.

In addition to breeding ecology, wildlife managers require an understanding of temporal segregation of subspecies and sex and the relative importance of migratory stop-over and wintering locations (e.g. migratory connectivity, Perrins et al. 1993). Studies elucidating migratory connectivity provide individual and population level information on what site-specific factors may be affecting over winter survival and help to identify cross-seasonal interactions (Webster et al. 2002). Differences in migration strategies, reproductive ecology, and physiological adaptations among subspecies and between sexes are documented across avian taxa (Gill 2007, Newton 2008, Cicero 2010, Winker 2010). Scientists use an increasingly assorted tool kit to classify subspecies including stable isotopes, morphometrics, and distinguishing genetics segments (e.g. mtDNA and microsatellite, Ball and Advise 1992, Haig et al. 2006). Advances in molecular sex determination methods allow for more accurate and precise sex determination that can be accomplished at a relatively low cost and investment in time (Griffiths et al. 1998, Jae-Ik et al. 2009). Studies incorporating sex and subspecies-specific estimates of survival, productivity, and migratory connectivity are greatly enhanced when individuals can be accurately classified.

To investigate renesting rates of Arctic-breeding birds, I selected Dunlin (*Calidris alpina*), because they are a model species for studying breeding ecology in Arctic ecosystems. In addition, they have a widespread Holarctic breeding distribution and are relatively abundant throughout their range (Hayman et al. 1986). Dunlin nest in a range of mesic tundra habitats, have a monogamous mating system, and a bi-parental incubation strategy (Warnock and Gill 1996). There are currently ten recognized subspecies of Dunlin including four subspecies breeding in the Nearctic (*C. a. arctica, arcticola, hudsonia*, and *pacifica*) and six subspecies breeding in the Palearctic (*C. a. actites, centralis, alpina, kistchinski, schinzii,* and *sakhalina*; Engelmoor and Roselaar 1998). During the breeding period, Dunlin subspecies are segregated across a broad geographic distribution. Conversely, multiple subspecies assemble at staging and

wintering locations across diverse global flyways, making it difficult to differentiate specific subspecies. North America populations of Dunlin (*C. a. arcticola, hudsonia,* and *pacifica*) are declining due to habitat loss and degradation mostly on the non-breeding grounds, and difficulties identifying critical staging and wintering areas for each subspecies confounds efforts to generate reliable populations estimates (Fernández et al. 2008).

In the second chapter of my thesis, I conducted a clutch removal experiment to document the capacity to replace lost clutches (i.e. renest) in Dunlin (*C. a. arcticola*). From 2007-2009, I individually color- and radio-marked approximately 20 pairs of Dunlin each year, and experimentally removed their entire clutches during two stages of the incubation period. Each marked pair was monitored to determine renesting propensity, number of days between nesting attempts (i.e. renest interval), mate fidelity, and clutch size and volume. Past observational studies have failed to measure renesting rates in a controlled setting where clutch loss could be experimentally manipulated to determine renesting rates under different incubation stages (Naves et al. 2008, Jamieson 2011). To my knowledge, this study is the first and only study using experimental approaches to investigate renesting ecology in Arctic-breeding shorebirds.

In the third chapter of my thesis, I used morphological and molecular approaches to determine sex and subspecies of Dunlin in order to generate better sex- and subspecies-specific demographic parameters. Considerable differences in ecology and physiology exist among the Dunlin subspecies and sexes; including their migration strategies, behavioral ecology, and physiological adaptations throughout their annual cycle (Colwell and Oring 1989, Shepherd et al. 2001, Silke 2007). The five subspecies of Dunlin breeding in Alaska and eastern Russia, referred to as "Beringia" Dunlin, can be challenging to separate due to overlap in morphometric and plumage characters. I used principal components and discriminant function analyses to evaluate if these techniques could be used to separate individuals into groups. I developed discriminant function models to (1) determine sex of all subspecies (e.g. *actites, arcticola, kistchinski, pacifica,*

sakhalina) and models to (2) determine subspecies at different staging/wintering locations where mixed subspecies aggregate.

CHAPTER 2: EXPERIMENTAL CLUTCH REMOVAL INDICATES HIGH RATE OF RENESTING IN AN ARCTIC-BREEDING SHOREBIRD.[1]

ABSTRACT

A bird's propensity to replace a clutch is a poorly understood and complex component of avian fecundity. Renesting rates differ greatly among avian taxa and vary with latitude and environmental conditions, female age, experience and physiological condition, food quality and availability, stage of incubation when clutch is lost, and date of nest loss. We removed clutches of an Arctic-breeding shorebird, Dunlin (*Calidris alpina arcticola*) during two distinct stages of incubation (early vs. late) to investigate (1) replacement clutch rates, renesting interval, mate fidelity, and movement between nesting attempts for females, (2) a female's initial and replacement investment in her clutches (e.g., clutch volume, mass, and egg size), and (3) factors affecting clutch replacement propensity. For the last objective, we examined the influence of male and female body condition, clutch volume, clutch initiation and loss dates, and year on renesting propensity. In contrast to prior studies in the Arctic, Dunlin had high replacement clutch rates (82-95%) after early clutch removal and moderate rates (35-50%) after late removal. Renesting intervals averaged 4.7-6.8 days across treatments and years. Mate retention was high (87-100%) and movements were short (< 100 m) between initial and replacement clutches. In the rare cases of divorce (n = 4), females moved considerable distances (>5 km) to renest with new males, while divorced males stayed on their initial territories and attempted to attract new females. The probability of renesting for female Dunlin was affected by female body condition and date the clutch was lost; larger females whose clutches were lost earlier in the season were more likely to replace their clutches than smaller females that lost their clutches later in the season. Studies focusing on Arctic shorebird breeding ecology should consider frequency and proportion of replacement clutches because the

[1] Gates, H. R., R. B. Lanctot, and A. N. Powell. Experimental clutch removal indicates high rate of renesting in Arctic-breeding shorebirds. Prepared for submission to Auk.

unaccounted presence of such nests leads to overestimates of nest density and underestimates of nest survival.

INTRODUCTION

Renesting propensity is a bird's ability to replace its clutch within the same season after a nesting attempt has failed due to predation and/or abandonment (Arnold 1993, Wiebe and Martin 1998). Renesting (i.e. clutch replacement) is one strategy employed by birds to compensate for clutch loss and can make important contributions to an individual's annual fecundity and lifetime reproductive success (Hipfner 2001, Morton 2002). Evolutionary and ecological constraints for renesting propensity are taxonomically and ecologically varied (Sandercock et al. 2005, Arnold et al. 2010). Species and populations subjected to high nest mortality rates have developed diverse strategies to compensate for disproportionate clutch loss (Fontaine and Martin 2006). Population estimates and demographic models most often lack information on renesting rates and survival of replacement clutches, despite the fact that renesting rate is an important component of fecundity (Arnold et al. 2010). A failure to understand the prevalence of renesting can also lead to underestimates of breeding population size, especially in situations where rapid surveys, which frequently limit observations to territorial birds, are used to assay shorebird presence and abundance (Bart and Earnst 2002, Skagen et al. 2003).

An individual's propensity to replace a clutch has been shown to be affected by female age, experience, and physiological condition (Grand and Flint 1996, Amat et al. 1999, Arnold et al. 2010); food quality and availability (Amat et al. 2001); stage of incubation when clutch is lost (Fondell et al. 2009); date of nest loss within the breeding season; latitude; and environmental conditions (Martin and Wiebe 2004, Sandercock et al. 2005). Female age and experience may positively contribute to renesting propensity presumably because older and more experienced migratory birds are in better physical condition upon arrival and nest earlier (Hipfner et al. 1997, Smith and Moore 2005). This

early nesting provides a longer window of opportunity for renesting under favorable weather conditions. Similarly, a reduced propensity to renest is likely to be affected by when a female loses her initial clutch, since at some point it should become ecologically impossible to lay and incubate a second clutch and successfully rear these offspring to fledging. A female's renesting propensity may also be influenced by her stage of egg follicle development, her physiological condition at clutch loss, and her ability to acquire sufficient food resources for egg production. As incubation progresses, levels of plasma luteinizing hormones decline and egg follicles become smaller, thus females that lose their clutch later in incubation will require more time to re-develop egg follicles (Donham et al. 1976, Williams 2005). Arctic-breeding shorebirds are income breeders and thus dependent upon local and seasonally abundant food resources to sustain energetic demands during the breeding period (Meijer and Drent 1999, Klaassen et al. 2001, Hobson and Jehl 2010). Egg production and clutch size are largely constrained by food availability and breeding strategy (Drent and Daan 1980). It also has been reported that species that breed at higher latitudes and elevations are less likely to renest due to a reduction in the length of the breeding and post-breeding seasons, and extreme weather conditions that make rearing offspring difficult (Johnson et al. 1992, Martin and Wiebe 2004, Sandercock et al. 2005). Moreover, long-distance migrants, including many of the Arctic-breeding birds, are further limited by temporal and reproductive inflexibility (Alerstam et al. 2003). While numerous explanatory factors have been examined across avian taxa, no single factor has been found to explain the variation in renesting propensity.

In the Arctic, a number of studies have reported moderate rates of replacement clutch laying in a variety of shorebird species (Holmes 1966a, Soikkeli 1967, Norton 1972, Schamel and Tracy 1977, Tulp 2007). However, these studies focused on general breeding biology of shorebirds and did not focus on clutch replacement. Moreover, in a long-term monitoring study in Barrow, Alaska, apparent replacement laying in shorebirds was reported as extremely low (< 1%; $n = 934$ nests), although renesting was detected in

five of 11 species monitored (Naves et al. 2008). Study design factors including the likelihood of finding first and replacement nests, timing of nest discovery, delayed or not marking of adults, and subsequent movement of renesting individuals outside the study area likely led to an underestimation of clutch replacement propensity (Naves et al. 2008).

We investigated renesting propensity by experimentally removing clutches and following individuals equipped with radio transmitters to directly document clutch replacement. We focused our work on an Arctic-breeding shorebird, the Dunlin (*Calidris alpina arcticola*), and removed clutches during two distinct stages of incubation (early and late). Based on previous work in the Arctic, we predicted that clutches removed in early incubation would have moderate replacement rates (<50%) and clutches removed later in incubation would be replaced at a low rate (<5%). We made further predictions that replacement clutches would be smaller in size and volume than initial clutches and that the number of days between clutch loss and initiation of replacement clutches would be longer for clutches removed during late incubation. We predicted that the majority of pairs would remain together in the event of clutch loss.

Herein, we present results on: (1) replacement clutch rates, renesting interval, mate fidelity, and movement between nesting attempts for females whose nests were removed both early and late in incubation, (2) a female's initial and replacement investment in her clutches (e.g., clutch volume, mass, and egg size), and (3) factors affecting clutch replacement propensity. For the last objective, we examined the influence of male and female body condition, clutch volume, clutch initiation and loss dates, and year on renesting propensity. Understanding what and how factors affect clutch replacement propensity in shorebirds will help us predict when replacement clutch laying will occur and how important it is to an individual's reproductive productivity and to subsequent population estimates in a given area.

METHODS

Study area

The study area was in the high Arctic, approximately 2 km south of the city of Barrow, Alaska (71.15°N, 156.48°W), adjacent to the Chukchi Sea in northern Alaska, USA, and encompassed approximately 10 km^2 of diverse tundra vegetation communities with xeric to mesic hydrologic conditions (Pitelka et al. 1974, Walker et al. 1980, Naves et al. 2008). Dunlin are an important component of a diverse avian breeding community, with densities averaging 18 nests/km^2 in nearby standardized plots (2007-2009, R. B. Lanctot unpubl. data). Snow cover is lost rapidly with the tundra becoming mostly snow-free by the first week of June. Mammalian predators in the study area included short-tailed and least weasel (*Mustela erminea* and *nivalis*), arctic fox (*Alopex lagopus*), and polar bear (*Ursus maritimus*); avian predators included Parasitic (*Stercorarius parsiticus*), Long-tailed (*S. longicaudus*), and Pomarine Jaegers (*S. pomarinus*); Glaucous Gull (*Larus hyperboreus*); Common Raven (*Corvus corax*); and Snowy Owl (*Nyctea scandiaca*). Beginning in 2005, arctic fox were removed from the Barrow area in the summer months to protect nesting Steller's Eiders (*Polysticta stelleri*) and their broods (USFWS 2002). Since arctic fox are one of the primary predators of shorebird nests (Liebezeit and Zack 2008, McKinnon and Bêty 2009), the lack of predators likely allowed us to find more initial and replacement nests then would have been possible otherwise.

Nest and clutch studies

We searched the study area intensively for Dunlin nests from early June to mid-July 2007-2009. We used behavioral signals and systematic nest searching techniques including delineating territories by the presence of singing and displaying males and then focusing search efforts within these areas; following adults back to their nests; flushing adults off of nests during area searches, and rope-dragging (Gratto-Trevor 2004). We attempted to minimize avian predation by conducting dummy nest checks at sites away from known nests during nest monitoring and trapping of adults to confuse predators. We

determined nest initiation dates from known laying dates, or by backdating to the onset of egg laying using egg flotation or known hatch dates, presuming a 21-day incubation period (Warnock and Gill 1996, Liebezeit et al. 2007).

Clutch removals and characteristics

We imposed two experimental clutch removal treatments by removing clutches during early (~2-8 days) and late (~12-16 days) stages of incubation. This allowed us to evaluate the impacts of clutch loss at different stages of a 21day incubation period. To control for nest initiation date, we used a systematic random approach to assign nests to the early or late clutch removal treatments as they were discovered. We removed entire clutches after both mates were successfully captured. In a few cases, the nest was depredated before both adults could be captured. Our study was approved by the University of Alaska Fairbanks, Institutional Animal Care and Use Committee (#08-12) and clutches were removed under U.S. Fish and Wildlife Service and Alaska State Fish and Game permits (MB088686-0, 08-122), respectively.

For all initial and replacement nests, we recorded clutch size and measured egg length (L) and breadth (B) to the nearest 0.1 mm with dial calipers. In addition, we obtained volume measurements for eggs (n = 23) via water displacement using the following equation: $K_v * L * B^2$ (K_v = Egg volume coefficient = 0.4735) (F. C. Governali unpubl. data, see also Hoyt 1979, Székely et al. 1994). We measured the mass of all eggs within clutches at the beginning of incubation (<1-2 days) to the nearest 0.01 g and female body mass to the nearest gram.

Marking and resighting adults

We used bownets to capture incubating adults on the nest. Both male and females were captured and uniquely marked with Darvic® color bands, a single green flag, and a U.S. Geological Survey aluminum leg band. We also attached a VHF radio transmitter (model BD-2, 1.4 g, Holohil Systems Ltd., Ontario, Canada) to each bird's back with Loctite® glue, approximately five mm above the uropygial gland (Warnock and Warnock

1993). Transmitters were adhered using an isopropyl alcohol accelerator before the Loctite® was applied in 2007 and 2008, but not in 2009.

We determined the sex of each bird using one of four methods (listed in descending order of preference): (1) molecular, (2) discriminant function analysis model, (3) presence of a distended cloaca, and (4) direct comparison of overall morphometric characteristics between two members of a pair. A description of the molecular techniques and discriminant function analysis are in Chapter Three. We used molecular sex of the individual or its mate for 86% (n = 161) of the individuals. We used a discriminant function equation (with an 86% classification accuracy) and morphometric measures to assign the sex of 10% (n = 19) of individuals (H. R. Gates unpubl. data). The presence of a distended cloaca aided us in determining the sex of one pair. When one or more of the measurements needed for the discriminant function equation were not available, we compared the culmen length, total head, wing and mass of both individuals of a pair and designated the individual with larger overall biometric measurements as female (Warnock and Gill 1996, Pyle 2008). This last approach was used for 3% (n = 5) of the individuals.

We waited three days after clutch removal before commencing radio-tracking activities to minimize disturbance. To relocate adults, find replacement clutches, and document mate and site fidelity, we tracked radio-marked adults every 1- 2 days using a hand-held three-element Yagi antenna and receiver (Advanced Telemetry Systems, Challenger R4000 model); individuals were monitored for up to 21 days after clutch removal. Pairs consistently observed during this period and never located at a new nest were categorized as non-renesters. Pairs that were seen irregularly or lost during the post-removal observation period were designated as unknown; these individuals either did not renest or moved beyond the search area to renest and were excluded from analysis.

During nest visits, we noted the identity of the marked adult on the nest, presence of the mate in the vicinity, nest stage (e.g. laying, incubation, or hatching), adult behavior (e.g. incubating, foraging) and checked nest contents. Adults were considered site faithful

if they nested within the initial territory boundaries of their first nest, and pairs were considered faithful to each other (i.e., exhibiting mate fidelity) if both members were either captured or observed departing the replacement nest. When pairs were apparently divorced an effort was made to locate both mates separately and confirm a new mate through resighting and capture. The presence of unique color band combinations allowed us to recognize individuals.

We conducted aerial telemetry surveys in early July each year, both within the study area and approximately 15 km beyond the study area, to search for birds that had not been detected during ground surveys. Locations determined from these surveys were subsequently visited on foot to locate the marked individuals and evaluate their renesting status.

Renesting, clutch, and pair characteristics

We summarized rates of replacement clutch laying for females and renesting by males separately to examine the participation of each sex in replacement clutches. We defined renesting interval as the number of days between clutch loss and the day the first egg was laid in the replacement clutch. Renest intervals were calculated separately for monogamous pairs versus male and female divorcees (n = 6 individuals). We analyzed clutch initiation dates and renest interval for monogamous pairs using a two-way analysis of variance (ANOVA) with year and clutch removal treatments as factors. We conducted post-hoc Tukey HSD multiple comparisons tests to examine significance differences between clutch removal groups ($\alpha = 0.05$).

We summarized the mean, standard deviation, and range of initial and replacement clutch size and clutch volume for monogamous and divorced pairs. We compared initial and replacement clutch size and volume for monogamous pairs using repeated measures two-factor ANOVAs, including clutch removal treatment and year as factors (Zar 1999). Clutch size and volume were analyzed separately for monogamous and divorced pairs.

We compared renest interval, clutch size, and clutch volume between initial and replacement nests for monogamous and divorced pairs. Due to low samples sizes for divorced pairs (~1 pair/year), we limited our comparison to descriptive statistics. We estimated distance from initial and replacement clutches by using a great-circle distance calculator (Schneider 2011).

Correlates of renesting propensity

We used logistic regression to examine the effect of year, initiation date, clutch loss date, number of days of incubation at clutch loss, female body condition, male body condition, and clutch volume on the probability of renesting. We calculated a body condition index score as body mass divided by wing chord as a proxy for an individual's current nutritional status (Piersma and Davidson 1991, Brown 1996). We imputed missing values using restricted maximum likelihood (REML) procedures for 30 cases of three factors: clutch volume, and male and female body condition indices (Little and Rubin 1987). Pairs that did not maintain their within-season pair bond were excluded from logistic regression analysis. We created 29 biologically relevant additive models that represented potential physiological, seasonal, or ecological constraints on renesting. We did not measure ecological constraints such as food resources directly, however, we included body condition and clutch volume because food availability is likely reflected in increased body condition and/or clutch volume. We calculated the Akaike Information Criterion value adjusted for sample size (AIC_c), and Akaike weights (w_i) to evaluate model support. The model with the lowest AIC_c value was considered the best and we defined ΔAIC_c as the difference between the AIC_c value of the current model and of the ΔAIC_c best model. We calculated model averaged parameter estimates, unconditional standard errors and 85% confidence intervals for models with $\Delta AIC_c \leq 4$ (Burnham and Anderson 1998, Anderson and Burnham 2002, Arnold 2010). We ranked the relative importance independent variables using cumulative sum of Akaike weights. Statistical analyses were conducted using JMP 8.0.2.

RESULTS

We removed 16-23 clutches during early incubation in 2007-2009, and 20 and nine clutches during late incubation in 2008 and 2009, respectively (Table 2.1). Nest initiation of experimentally removed clutches occurred during the first two weeks of June in all years (Table 2.1); dates were significantly different among years ($F_{2, 85} = 18.472$, $P < 0.0001$) but not between treatments (i.e., nests that had clutches removed early and late in incubation were initiated at roughly the same time, $F_{1, 85} = 0.335$, $P = 0.564$). Post-hoc Tukey HSD multiple comparison tests indicated that initiation dates in 2009 were earlier than 2007 and 2008 ($q_{0.05} = 2.385$). Nest age at removal averaged five days and 13 days for early and late clutch removal, respectively (Table 2.1).

Renesting, clutch, and pair characteristics

From 2007-2009, initial clutch initiation dates were between late May and early June, while initiation dates of replacement clutches were mid to late June (Fig. 2.1). The high rates of renesting yielded a bi-modal distribution of initiation dates (Fig. 2.1). Dunlin had high (82-95%) and more moderate (35-50%) replacement clutch rates after early and late clutch removal, respectively (Table 2.2). Renesting intervals averaged < 1 week for both clutch removal treatments, and ranged between 2-15 days for early removal and 3-8 days for late removal (Table 2.2). Renesting intervals were not different among years ($F_{2,53} = 2.60$, $P = 0.08$) or between clutch removal treatments ($F_{1, 53} = 2.28$, $P = 0.13$). Renesting intervals were 5.3 ± 1.9 d (range 4-8, $n = 4$) for divorced females and 8.0 ± 4.2 d (range 5-11, $n = 2$) for divorced males.

We found no differences in clutch size among years. All initial nests had 4-egg clutches, however, 14% of early replacement clutches contained ≤3 eggs, and 27% of late removal replacement clutches contained ≤3 eggs (Table 2.3, $F_{1, 40} = 7.99$, $P < 0.01$). For divorcees, clutch size remained consistent between initial and replacement clutches with 4-eggs per clutch. We found no difference in clutch volume among years, but clutch volume was smaller in replacement clutches compared to initial clutches ($F_{1, 40} = 14.76$, $P = 0.04$). The reduction in clutch volume was more pronounced in late replacement

clutches when compared with replacement clutches from the early removal treatment ($F_{1, 40} = 4.99$, $P = 0.04$).

The average mass of female Dunlin captured during our study was 62.3 ± 3.1 g, the average mass of a fresh 4-egg clutch was 47.6 ± 2.3 g, and the average mass of a single egg was 11.9 ± 0.6 g. Thus, an entire clutch of eggs represents 76.5 ± 4.6% of a female's total body mass and a single egg represents 19.1 ± 1.1% of a female's body mass. Clutch volume for male divorcees was 47.3 ± 1.2 cm^3 ($n = 2$) and 45.3 ± 0.9 cm^3 ($n = 4$) for female divorcees.

Dunlin pairs exhibited strong mate fidelity (>87%) after clutch loss; most pairs remained together to lay and incubate a replacement clutch within 100-300 m of their initial nest (Table 2.2). Divorce rates were low (<13%) for early clutch removal treatment and no pairs divorced in the late removal treatment (Table 2.2). When divorce occurred, males remained on their initial territory and nested again or attempted to attract a new female (Fig. 2.2, $n = 2$, 259 ± 197 m, range = 120-398 m), while divorced females moved greater distances to find a new mate and nest again (Fig. 2.2, $n = 4$, 5.3 ± 2.8 km, 2.0-8.1 km).

Eight individuals comprising four breeding pairs, and eight other individuals belonging to different pairs, returned to the study area to breed in multiple years and were subjected to different clutch removal treatments among years. In spite of removal treatment, the four pairs replaced their clutches 89% of the time, whereas returning individuals that did not retain between-season pair bonds replaced their clutches 55% of the time. One pair returned all three years of the study and replaced clutches each year. All returning individuals who failed to replace their clutches after clutch removal were part of the late removal treatment group.

Factors affecting replacement clutches

Renesting propensity was positively affected by a female's body condition ($\beta_{FC} = 26.71$) and negatively affected by clutch loss date ($\beta_{CL} = -0.209$, Table 2.4, Fig. 2.3). Male body condition was inversely related to renesting propensity, with smaller males

more likely to renest than larger males (β_{MC} = -6.818). Female body condition was the only factor found in all eight top models (sum of AIC weights = 0.88, Table 2.5, Fig. 2.4). Date of clutch loss also had a high sum weight (0.64, Fig. 2.4). Male body condition, initiation date, clutch volume and year had lower relative importance and 85% confidence intervals including zero, indicating that they are uninformative.

DISCUSSION

Contrary to our expectations, Dunlin renesting probability was high, between 82%-95% when clutches were removed during early incubation, and 35%-50% when removed during late incubation. This is surprising given the energetic costs of laying two clutches, with females investing approximately 70-80% of their body mass per clutch and breeding at high latitudes. Moreover, Dunlin undergo flight feather molt during early incubation, further increasing already considerable energetic demands during the breeding period (Holmes 1971). In addition, even with our experimental approach and use of radio transmitters to follow individuals, our replacement rates were likely conservative because of radio loss, birds potentially moving beyond the study area, and depredation of replacement nests before discovery. Thus, our estimates represent a minimum renesting propensity based on observational data.

Although laying of replacement clutches has been documented in Arctic and Subarctic breeding shorebirds with monogamous mating systems (Soikkeli 1967, Tulp 2007, Johnson et al. 2008, Jamieson 2011), anecdotal data from other studies reported rates that were considerably lower than what we documented (Naves et al. 2008). In addition, paternity studies have documented individual females of polyandrous species laying multiple clutches in a year (Schamel and Tracy 1977, Dale et al. 1999, Schamel et al. 2004a, Schamel et al. 2004b). Thus, other Arctic and Subarctic species likely have much higher renesting propensities than previously thought. Unfortunately no other studies have focused on documenting rates of renesting in Arctic-breeding shorebirds, nor

have any studies used experimental techniques to assess what factors influence renesting rates.

Although our results indicate that clutch replacement is a common reproductive compensation strategy employed by Dunlin to increase reproductive success, the ability to replace nests appears to be constrained by several factors. We found that female body condition was the most important variable influencing the propensity to renest; females in better body condition were more likely to replace a clutch, suggesting a female's current nutritional status plays a key role in her propensity to compensate for clutch loss by renesting. The date of clutch loss was an important determinant of whether a female will replace her clutch. This was apparent when renesting rates were compared between our early and late removal treatment groups. A study examining the renesting ecology of Kentish Plovers (*Charadrius alexandrinus)* found individuals were primarily constrained by timing of nest initiation; however, they did not examine timing of clutch loss (Amat et al. 1999). We included both measures of timing (nest initiation and clutch loss) and found that timing of clutch loss was more important than nest initiation date.

It was also surprising that females laid second clutches in more than one year of our study. In fact, individuals that showed breeding site fidelity renested at equally high rates as those captured on the study area for the first time. This repeated renesting in subsequent years suggests that reproductive compensation via renesting is a persistent characteristic; however, it remains unclear if replacement clutch laying has a negative impact on survival or other reproductive parameters (e.g. nest initiation). Amat et al. (1999) examined multiple-year renesting for Kentish Plovers and found individuals did not delay breeding or breed less frequently after laying replacement clutches in previous years. Dunlin's ability to replace clutches suggests they have a flexible energy budget in spite of challenging ecological conditions on the breeding grounds.

The interval length between clutch loss and initiation of replacement nests was shorter in our study compared to other studies. Renesting intervals for a Subarctic-breeding population of Dunlin (*C. a. pacifica*) averaged 9.3 days (Jamieson 2011),

approximately three days longer than our high Arctic population. Moreover, Pacific Golden-plovers (*Pluvialis fulva*) breeding at Subarctic latitudes replaced clutches approximately one week after clutch loss (Johnson et al. 2008). The shorter renest interval found in our Arctic-breeding population likely represents physiological adaptations (e.g. rapid egg-follicle growth, high metabolism) maintained by females to rapidly respond to predation (Williams 2005). Higher energy expenditure has been found in Arctic- versus temperate-breeding shorebirds (Piersma et al. 2003), which may explain why renest interval between Arctic and Subarctic populations are notably different. High energy expenditure has also been found in Arctic shorebird chicks resulting in faster development rates and better cold hardiness than their temperate counterparts (Schekkerman et al. 2003). Arctic breeding females may be adapted to develop egg follicles at a quicker rate due to increased energy expenditure capabilities.

Clutch volume was reduced between initial and replacement clutches in our study. Late replacement clutch volume was reduced more than early replacement clutches denoting a seasonal decline in clutch volume investment. Moreover, clutch size was also reduced in replacement clutches; however, there was no difference between early and late replacement clutches. Evidence from food availability and abundance studies in Barrow have revealed that food abundance is relatively high (~ 900 mg dry weight/m^2) and shows a bi-modal peak in mid-June and early August (Holmes 1966b). Taking into consideration our high renesting rates, it appears that local food resources and nutrients (e.g. carotenoids and calcium) were not limiting replacement clutch propensity, but were limiting investment in replacement clutches, especially during the late season.

Dunlin pairs subjected to clutch loss demonstrated strong monogamous pair bonds and renested in close proximity to their initial nest. The low divorce rates we found suggest that either the costs of divorce outweigh the potential benefits of finding a new mate (Ens et al. 1996, Van De Pol et al. 2006), or that new mates were not available. The latter seems possible given that no divorce occurred in the late removal treatment when it was expected that most adults would have been occupied in nesting efforts. In the few

instances where females divorced their mate, a consistent pattern emerged with females moving outside of their initial nesting territory and renesting with new males. Conversely, divorced males remained on their territory and nested with a new female. Due to the compressed breeding period in the Arctic, it seems likely that there is a competitive advantage in maintaining the within-season pair bond due to the time required to find and court a new mate. However, renest intervals of divorcees was similar to monogamous pairs, demonstrating that divorce did not result in a reduction in time between clutches.

Replacement clutches may compensate for a proportion of first-nest losses (Parker 1985, Amat et al. 1999) and make important contributions to a species' annual productivity and to an individual's lifetime reproductive success, especially in areas with high egg mortality (Hipfner 2001, Morton 2002). Our results demonstrate that clutch replacement is a frequent reproductive compensation response for Dunlin and is therefore an important parameter to include in productivity estimates. A way to estimate rates of renesting might be to examine initiation dates of a species at a given site and look for a distinctive bimodal distribution. Each bimodal peak could be used to identify the relative frequency and timing of initial and replacement clutches. For example, Dunlin nests initiated in our study area between 31 May and 12 June would most likely be initial nests, whereas nests initiated between 14 June and 2 July would most likely be replacement nests (Fig. 2.1a-c). Misleading estimates of fecundity may be obtained if replacement clutches are not taken into account (Green and Hirons 1988, Thompson et al. 2001, Frederick et al. 2006).

Another reason to document renesting is to allow more accurate estimates of nest density. An example of this issue can be illustrated by the Program for Regional and International Shorebird Monitoring (PRISM), which launched an effort in 2001 to estimate the size of North America's shorebird breeding populations (Bart et al. 2005). Methods employed by PRISM include double sampling with both rapid and intensive surveys. Intensive surveys are conducted with the purpose of determining the true

number of breeding pairs on a plot (Bart and Earnst 2002, Bart et al. 2005). The intensive surveys visit 400-m^2 plots between early June and early July. Rapid surveys later visit these plots a single time and estimate number of pairs. The ratio of these two estimates are used to correct the rapid survey density estimates. Given the high rates of replacement clutch laying in this study, the fact that the area surveyed by PRISM plots is sufficiently large that an individual bird could lay both an initial and replacement clutch within a single plot, and because birds are not uniquely marked during PRISM surveys, it is likely that a portion of the discovered nests are replacement clutches, which if not differentiated would lead to an overestimate in the numbers of breeding birds. This would be particularly problematic in years where predation rates are high and birds are likely to lay replacement nests at high rates. To rectify this problem we suggest removing nests initiated later in the season when estimating nest density or number of breeding pairs.

Renesting rates are a poorly understood yet important component of demographic studies and productivity estimates. Further studies are necessary to examine physiological constraints, the influence of timing of clutch loss and how ecological resources limit renesting. Examination of diverse shorebird species and other Arctic breeding birds at different latitudes will yield a better understanding of how widespread and at what rates renesting occurs. And ultimately allow an evaluation of the ecological and evolutionary drivers of avian breeding ecology in northern latitudes.

ACKNOWLEDGMENTS

We thank Joaquin Aldabe, Noah Burrell, Chung Yu Chiang, Jimmy Choi, Jen Coffey, Charles Governali, Ben Haase, Brooke Hill, Jean-Francois Lamarre, Bert Lewis, Pete Mickelson, Robert Rankin, and Audrey Taylor for diligent field assistance. Liliana Naves, Christine Hunter, Brett Sandercock, Joel Schmutz, Stephen Yezerinac, and Kevin Winker offered keen insights throughout the development of this project. Dana Thomas offered statistical advice. Aerial survey pilots from Arctic Air Alaska, Robert Bird and Bob Eubank, and U.S. Fish and Wildlife Service pilot/biologist, Karen Bollinger skillfully navigated flights in the Barrow airspace. This work has greatly benefitted from the thoughtful reviews of Brooke Hill and Bert Lewis. Funding and logistical support was provided by Bureau of Land Management, Arctic Field Office; US Fish and Wildlife Service Migratory Bird Management Program; U.S. Geological Survey, Alaska Cooperative Fish and Wildlife Research; University of Alaska Fairbanks; and Arctic Audubon. The use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by either the US Government.

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Table 2.1. Initiation dates and nest age at removal (mean ± SD, range in parentheses) for Dunlin (*Calidris alpina arcticola*) clutches that were experimentally removed early (2007-2009) or late (2008-2009) in the incubation period near Barrow, Alaska, USA.

	Early removal			Late removal	
	2007	2008	2009	2008	2009
	n = 16	*n* = 21	*n* = 23	*n* = 20	*n* = 9
Clutch initiation date	7 June ± 1.3d (6 – 11 June)	6 June ± 2.1d (3 - 12 June)	5 June ± 1.8d (2 – 8 June)	7 June ± 2.2d (3 -12 June)	3 June ± 2.4d (31 May - 8 June)
Nest age at removal (days)	4.8 ± 1.7d (2 – 7)	5.2 ± 1.4d (3 – 8)	5.6 ± 1.5d (3 – 8)	13.0 ± 1.2d (12 – 16)	13.0 ± 1.2d (12 – 15)

n refers to number of nests in each category.

Table 2.2. Replacement clutch, renesting and divorce rates, renest interval (mean ± SD, range in parentheses), distance to replacement clutch (mean ± SD) for Dunlin (*Calidris alpina arcticola*) pairs near Barrow, Alaska, USA whose clutches were experimentally removed early (2007-2009) or late (2007-2009) in the incubation period. Renest interval and distance between initial and replacement clutches are reported for between - clutch monogamous pairs only.

	Early removal				Late removal		
	2007	2008	2009	All years	2008	2009	All years
	n = 12	*n* = 18	*n* = 17	*n* = 47	*n* = 7	*n* = 4	*n* = 11
Replacement clutch rate, females (%)	85	95	82	87	35	50	43
Renesting rate, males (%)	89	81	85	85	35	30	33
Divorce rate (%)	6	5	13	8	0	0	0
Renest interval (days)	6.8 ± 2.9 (5 – 15)	4.7 ± 1.5 (2 – 7)	6.4 ± 3.0 (2 – 14)	6.0 ± 2.5 (2 - 15)	6.6 ± 1.6 (4 – 8)	5.3 ± 2.1 (3 – 7)	6.5 ± 3.0 (3 - 8)
Distance between initial & replacement nests (m)	232 ± 126	161 ± 113	185 ± 168	192 ± 136	187 ± 141	201 ± 80	194 ± 111

n refers to number of nests in each category.

Table 2.3. Clutch size (mean ± SD) and clutch volume (mean ± SD) for Dunlin (*Calidris alpina arcticola*) whose clutches were experimentally removed early (2007-2009) or late (2007-2009) in the incubation period near Barrow, Alaska, USA.

	Early removal						Late removal			
	2007		2008		2009		2008		2009	
	Initial $n = 8$	Renest $n = 7$	Initial $n = 16$	Renest $n = 15$	Initial $n = 15$	Renest $n = 13$	Initial $n = 6$	Renest $n = 6$	Initial $n = 3$	Renest $n = 3$
Clutch size	4.0	3.9 ± 0.4	4.0	3.9 ± 0.2	4.0	3.8 ± 0.4	4.0	3.7 ± 0.5	4.0	3.7 ± 0.6
Clutch volume (cm^3)	44.6 ± 2.5	42.7 ± 6.0	45.5 ± 2.1	44.2 ± 4.7	46.1 ± 2.1	42.7 ± 5.3	47.2 ± 2.7	39.1 ± 5.7	43.2 ± 2.9	38.9 ± 5.0

Table 2.4. Logistic regression models assessing factors affecting the probability of Dunlin (*Calidris alpina arcticola*) laying a replacement clutch in Barrow, Alaska, USA 2007-2009. All models with Δ $AIC_c \leq 4$ are shown.

Model	K	*Log* Likelihood	AIC_c	ΔAIC_c	w_i	$\sum_r w_i$
FC + JL	3	-44.878	96.039	0.000	0.289	0.289
FC + JI + DI	4	-44.631	97.737	1.698	0.124	0.413
FC + JL + MC	4	-44.712	97.900	1.861	0.114	0.527
FC + JL + CV	4	-44.840	98.157	2.118	0.100	0.627
FC + JL + MC + YE	5	-43.811	98.344	2.305	0.091	0.718
FC + DI	3	-46.140	98.562	2.523	0.082	0.800
FC + JL + JI + MC	5	-44.572	99.867	3.828	0.043	0.843
FC + JI + DI + CV	5	-44.598	99.919	3.880	0.042	0.884

K is the number of parameters, and w_i is the Akaike model weight. Abbreviations in model descriptions are: FC = index of female body condition, JL = Julian date of clutch removal/loss, JI = Julian initiation date of initial clutch, DI = number of days of incubation at clutch loss, MC = index of male body condition, CV = clutch volume, YE = year.

Table 2.5. Logistic regression model parameters, constraint type (i.e. physiological, seasonal, ecological), relative variable importance (summed Akaike weights), model averaged parameter estimates (all models with $AIC_c \leq 4$), standard errors (SE), and 85% confidence intervals for the probability of Dunlin renesting after clutch removal in Barrow, Alaska USA, 2007-2009.

					85% confidence intervals	
Variable	**Constraint type**	**Relative variable importance**	**Model averaged parameter estimate**	**SE**	**Lower**	**Upper**
Intercept			19.002	15.378	41.146	-3.143
Female body condition	Physiological and ecological	0.88	26.713	10.413	41.708	11.719
Clutch loss date	Seasonal	0.64	-0.209	0.064	-0.117	-0.302
Male body condition	Physiological and ecological	0.25	-6.818	11.108	9.177	-22.813
Days of incubation at clutch loss	Physiological and seasonal	0.25	-0.211	0.066	-0.116	-0.306
Julian initiation date	Seasonal	0.21	-0.122	0.132	0.068	-0.312
Clutch volume	Physiological and ecological	0.14	0.00003	0.0001	0.0002	-0.0001
2007	Seasonal	0.09	0.297	0.509	1.030	-0.435
2008	Seasonal	0.09	0.247	0.468	0.922	-0.427

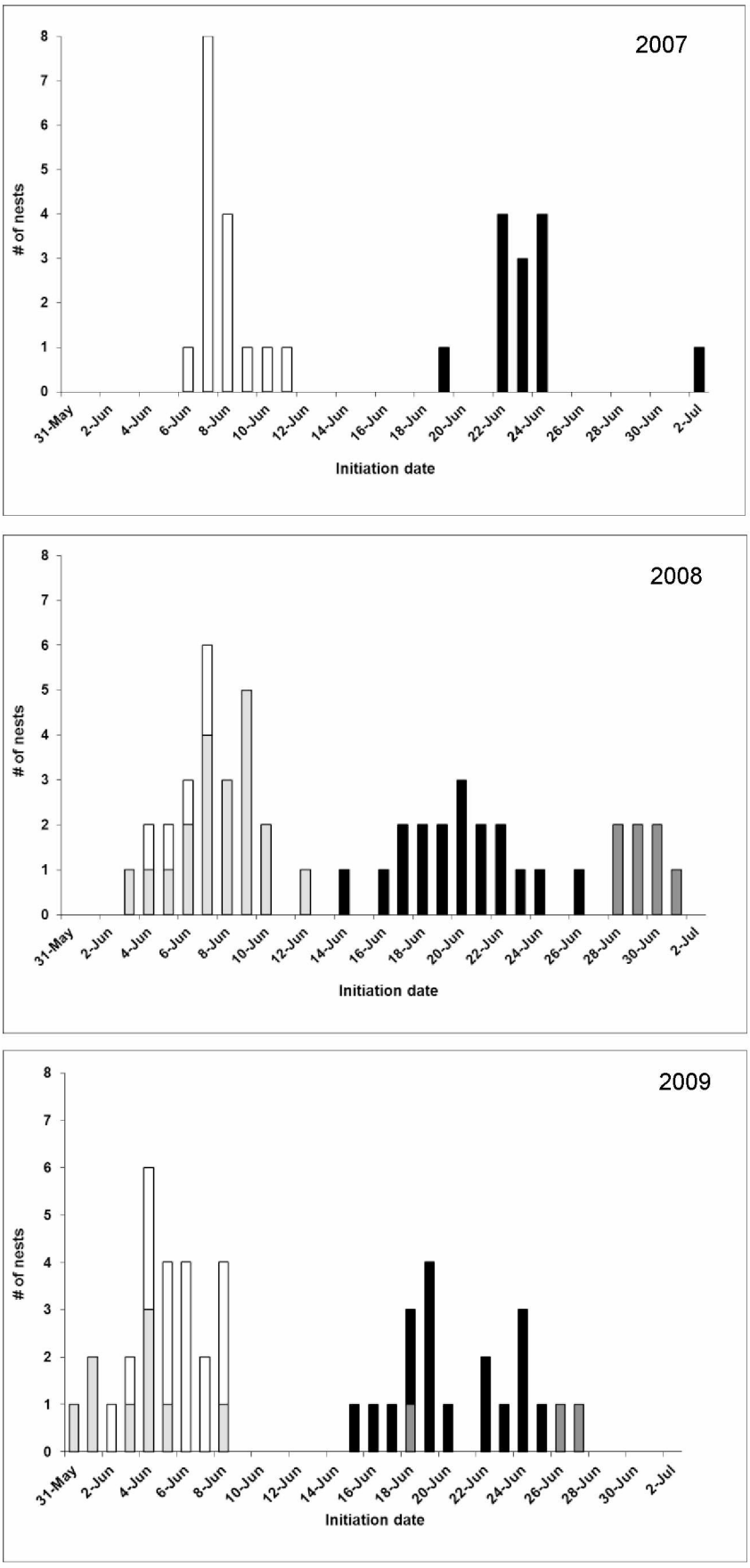

Figure 2.1. Nest initiation dates of Dunlin (*Calidris alpina arcticola*) for experimentally removed initial clutches (early: white; late: light gray) and replacement clutches (early: black; late: dark gray) from 2007-2009 at Barrow, Alaska, USA.

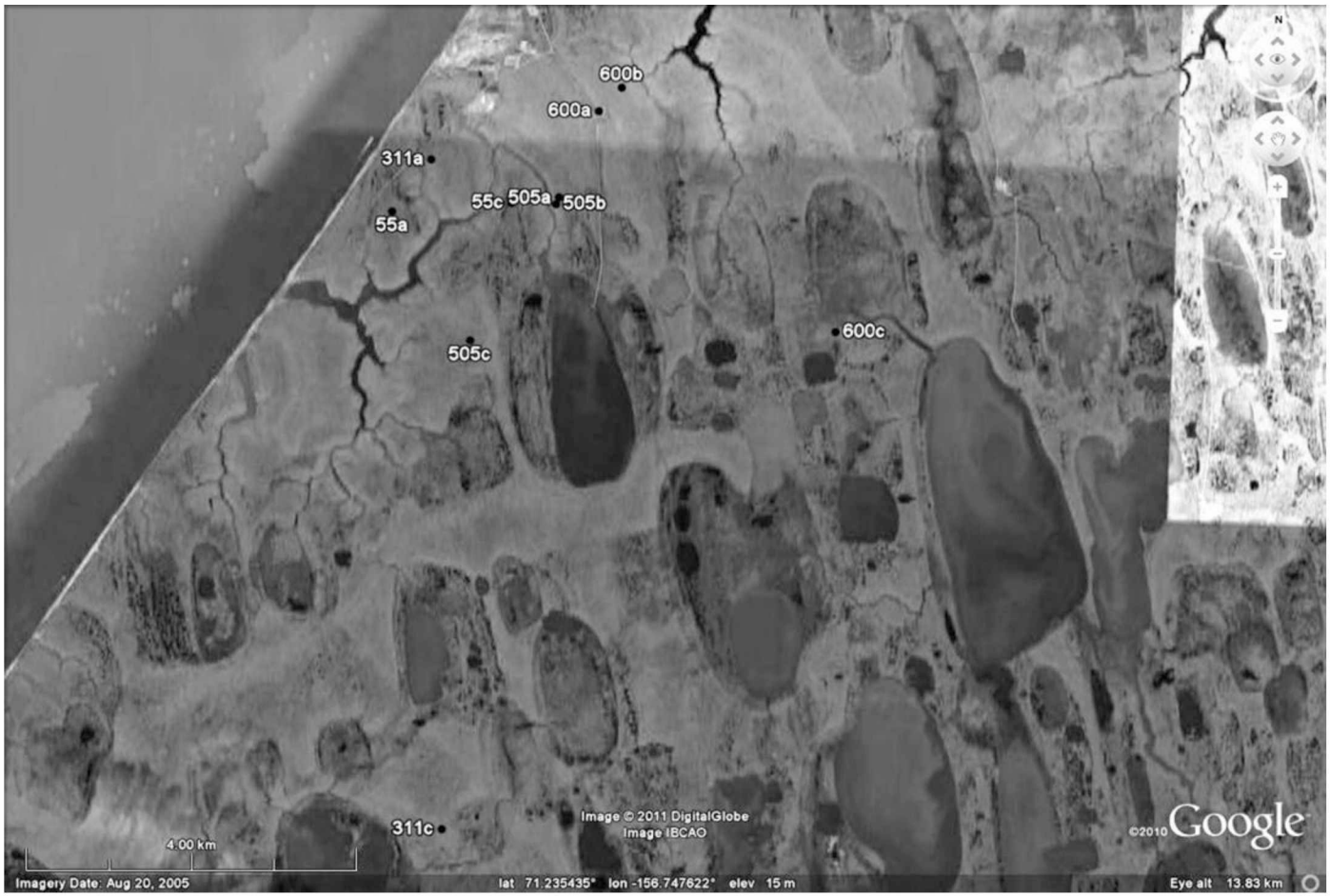

Figure 2.2: Unique pair identification codes (e.g. 55, 311, 505, 600) and locations of initial clutch ("a") , divorced male clutch ("b"), and divorce female replacement clutch ("c") of Dunlin (*Calidris alpina arcticola*), Barrow, Alaska 2007-2009.

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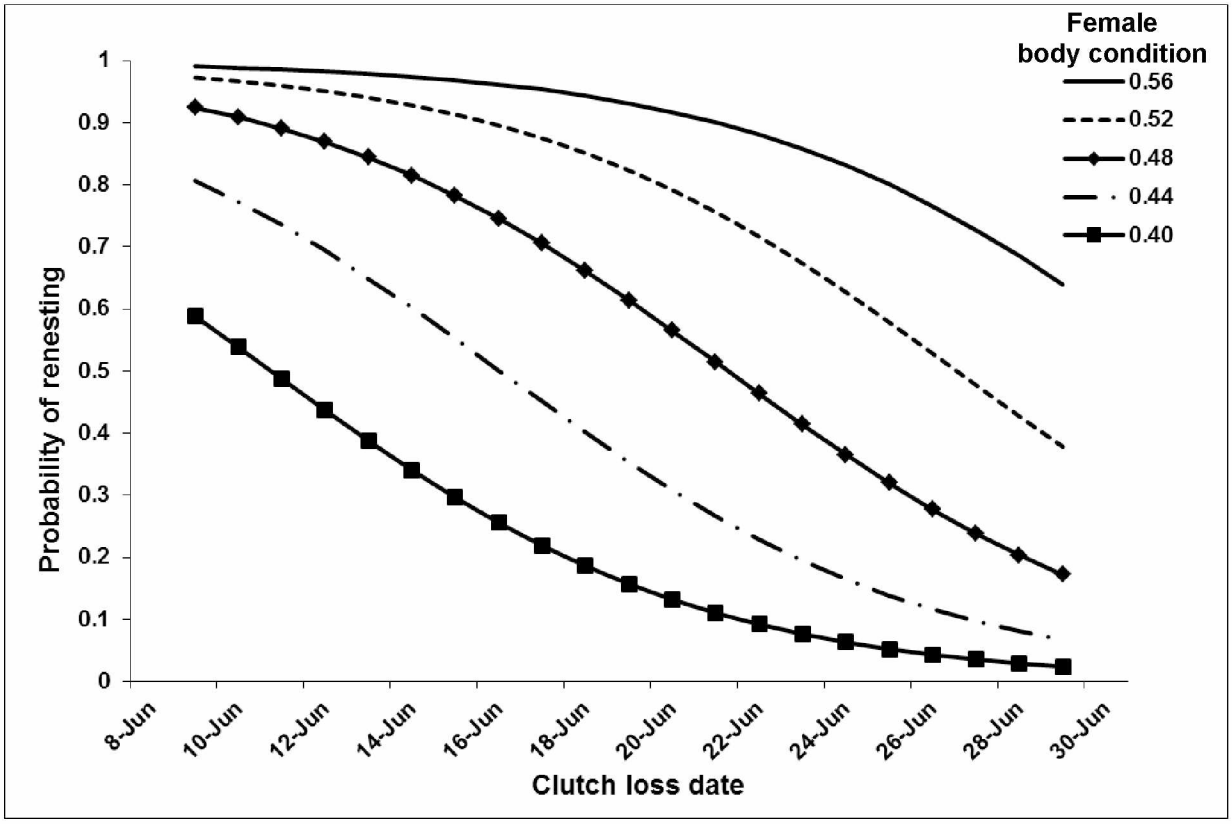

Figure 2.3. Probability of renesting for Dunlin (*Calidris alpina arcticola*) given clutch loss date and female body condition, at Barrow, Alaska, USA, 2007-2009.

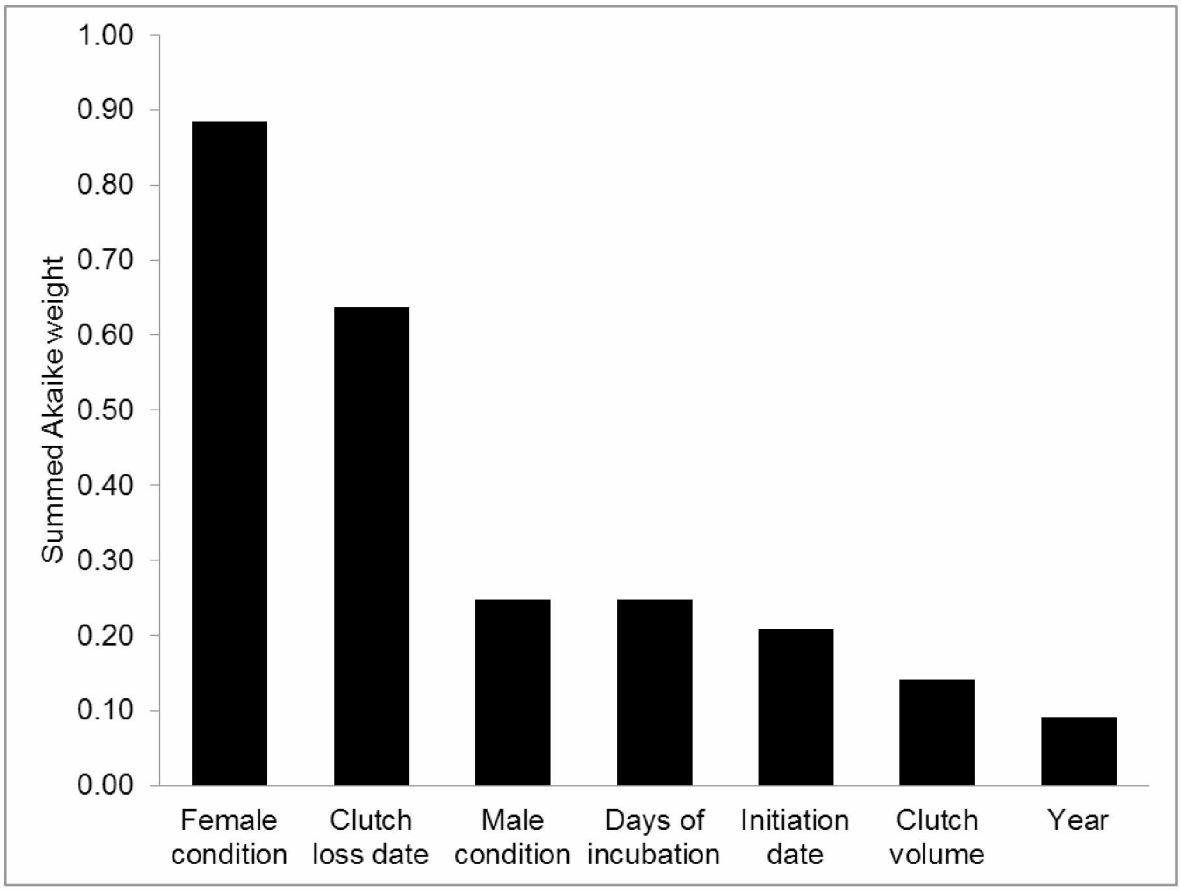

Figure 2.4. Summed Akaike weights for logistic regression model parameters for all models with $AIC_c \leq 4$ for the probability of Dunlin renesting after clutch removal in Barrow, Alaska USA, 2007-2009.

CHAPTER 3: MORPHOMETRIC TECHNIQUES FOR DIFFERENTIATING SUBSPECIES AND SEX OF BERINGIA DUNLIN [1]

ABSTRACT

Development of tools for delineating subspecies and sex of birds is useful for determining composition of mixed assemblages at non-breeding aggregation sites. We investigated the possibility of using morphological measurements (e.g. exposed culmen, total head, tarsus, wing and body mass) to differentiate five subspecies of Dunlin (*Calidris alpina actites, arcticola, kistchinski, pacifica,* and *sakhalina*) that breed in Beringia and have overlapping migration and wintering sites. Differences in subspecies were explored using principal components and discriminant function analysis where the sex of individuals was and was not known. We also developed discriminant function models to determine sex of individuals for each subspecies using individuals sexed with molecular techniques. Discriminant function models classified subspecies that overlapped at different geographic locations correctly 55-73% of the time. Correct classification of mixed subspecies assemblages improved when sex was first determined via molecular techniques. Not surprisingly, subspecies more similar in size were more difficult to differentiate. Discriminant function models were able to differentiate the sex of individuals of known subspecies 86-96% of the time; a classification rate useful in most field situations. Our efforts to separate subspecies were likely reduced because we did not incorporate plumage traits into our analysis that had been previously used to describe subspecies. Our analysis, which was based on simple field measurements, represents a logical first step for separating subspecies given the inherent variation in plumage among individuals on the breeding grounds, the lack of such distinguishing traits on birds at nonbreeding sites, and difficulties in measuring plumage coloration accurately. Incorporating molecular sex differentiation helped us differentiate subspecies, but requires additional work outside of the field. It remains to be seen whether on-going

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[1] Gates, H. R., S. Yezerinac, R. B. Lanctot, A. N. Powell, P. S. Tomkovich, O. P. Valchuk. Morphometric techniques for differentiating subspecies and sex of Beringia Dunlin. Prepared for submission to Condor.

genetic and stable isotopic techniques can better differentiate these subspecies during various stages of the annual cycle.

INTRODUCTION

Dunlin (*Calidris alpina*) are long-distance migratory shorebirds with a Holarctic breeding distribution and complex global migration pathways (Warnock and Gill 1996, Message and Taylor 2005). The species' complex worldwide geographic distribution and numerous (8-10) subspecies have prompted several studies investigating their taxonomy (Browning 1971, Greenwood 1986, Tomkovich 1986, Nechaev and Tomkovich 1987, Browning 1991, Engelmoor and Roselaar 1998, Marthinsen et al. 2007). The Beringian Dunlin are a group of five subspecies (e.g. *C. a. actites, arcticola, kistchinski, pacifica, sakhalina*) that breed in the geographic area of northern and western Alaska, northeastern Siberia, and the Bering Strait. There are two Nearctic subspecies including *arcticola* that breed on the Arctic Coastal Plain of Alaska, and *pacifica* that breed in coastal western Alaska. Three subspecies breed in the eastern Palearctic, including *sakhalina* in northeastern Siberia, *kistchinski* on the Kamchatka Peninsula in southern Siberia and *actites* on *Sakhalina* Island north of Japan (Fig. 3.1).

These Dunlin subspecies overlap at migration and wintering sites both in Asia and Alaska, and present challenges because of their complex range overlap throughout the annual cycle. The *arcticola* and *pacifica* subspecies stage in Western Alaska (e.g. Yukon Kuskokwim Delta) prior to migrating along the Asian-Australasian Flyway (*arcticola*) and the Pacific Flyway *(pacifica*), respectively (Warnock and Gill 1996). The *arcticola* subspecies winters in Japan, Taiwan, North and South Korea, and the People's Republic of China where it likely overlaps with the three Russian subspecies (*actites*, *kistchinski* and *sakhalina*) (Lanctot et al. 2009). At present, known migration routes and stopover locations are based on a limited number of resightings and recaptures of banded birds (Warnock and Gill 1996, Lanctot et al. 2009). The distribution of birds based on resighting and recaptures are likely incorrect or at least incomplete, due to observers not

being evenly distributed throughout the wintering range. Understanding how these subspecies are distributed in space and time is important for conservation, especially for subspecies with small populations (e.g. *actites*) that may be disproportionally affected by negative impacts, or situations where documenting exposure to pathogens is important (e.g. *arcticola* and H5N1, avian influenza).

Historically, subspecies were identified based on morphological measurements, alternate plumage characteristics, and geographic extent during the breeding season (Winker 2010). More recently, genetic phylogeographic studies have been used to verify the validity of subspecies designations according to currently accepted standards. The reduced number of phylogeographic groups recognized by molecular methods may in part be because enough time has not passed for the accumulation of new mutational differences that result in distinguishable mtDNA loci or genetic markers (Winker 2010, Buehler and Baker 2005). In addition, most Dunlin subspecies have overlapping breeding ranges across their Holarctic distribution (Fig. 3.1) further reducing genetic isolation (Wennerberg 2001). For example, Wenink et al. (1996), using mtDNA, identified only five phylogeographic groups for Dunlin. However, molecular techniques are costly and time-consuming, and it remains untested if morphological measurements alone can be used to distinguish the Beringian subspecies. Herein we test whether morphological measurements could be used to separate these subspecies. We chose not to use plumage characteristics, although they are frequently used to differentiate subspecies, because of the limited applicability to field studies when birds are not in alternate plumage (e.g. at wintering and staging sites) and when they congregate in mixed subspecies flocks.

The objectives of this study were to use morphometric measures (specifically exposed culmen, total head, tarsus, flat straight wing and body mass) of male and female adults of five Beringian Dunlin subspecies to 1) describe the degree of sexual dimorphism in each subspecies, and 2) assess the utility of using morphometrics measures for classifying individuals to either sex or subspecies. We limited analyses to differentiate subspecies to those groups thought to overlap at migration stopover sites.

METHODS

Study areas

We captured or collected individual adult Dunlin from 10 breeding populations representing five subspecies, including three subspecies in eastern Siberia, Russia, and two in Alaska, USA (Fig. 3.2). The *actites* subspecies was captured or collected at Chaivo Bay, Sakhalin Island, Russia (Fig. 3.2, site 1: 52°31'N, -143°17'W) between 2007 and 2009. The *kistchinski* subspecies was collected or captured at two locations on the Kamchatka Peninsula, Russia: Southwest (Fig. 3.2, site 2: 52°48'N, -156° 25'W) in 2009 and Fchun (Fig. 3. 2, site 3: 56°30'N, 155°59'E) in 1989. The *sakhalina* subspecies was captured or collected at four locations in Russia: Khatyr (Fig. 3.2, site 4: 62° 7' N, 175° 26'E), Meinypilgno (Fig. 3.2, site 5: 62°31'N, 177°1'E), Vtoraya (Fig. 3.2, site 6: 64°22'N, 177°25'E) and Belayaka (Fig. 3.2, site 7: 67°3'N, 174° 37'E), between 1986 and 2009. The *arcticola* subspecies was captured at Point Barrow, Alaska, USA (Fig. 3.2, site 8: 71°14'N, -156° 33'W) between 2003 and 2009. Finally, the *pacifica* subspecies was captured on the Yukon-Kuskokwim Delta, Alaska, USA at two locations, Manokinak River (Fig. 3.2, site 9: 61° 11'N, -165° 5'W), and Platinum Spit (Fig. 3.2, site 10: 59° 1'N, -161°49'W) in 2009. Capture and/or collection of individuals at all sites was conducted during the breeding season, between mid-June and mid- July.

Field methods

The majority of individuals belonging to the *actites*, *kistchinski*, and *sakhalina* subspecies were collected; measurements were obtained from dead birds within 24 hours. In contrast, individuals from *pacifica* and *arcticola* subspecies were captured live on their nests using bownets and walk-in traps and were released after measurements were taken (Gratto-Trevor 2004). We used dial calipers to measure exposed culmen, total head, and diagonal tarsus to the nearest 0.1 mm. We measured flat straightened wing length to the nearest 0.5 mm. However, *actites* wings were not straightened prior to measuring wing chord. To make these measures commensurate, we built a linear regression model using paired flat straightened wing and flat wing measures of *sakhalina* birds (flat straight wing

= 28.115 + 0.808 * flat wing, $r^2 = 0.75$, n = 88, Tomkovich unpubl. data) and used this to adjust the *actites* wing measurements. We recorded mass to the nearest 1.0 g using a 100 g Pesola® scale. One or a few people with experience in taking shorebird morphological measurements collected data at each location.

We extracted blood (10-50 µl) from the brachial vein using a capillary tube and/or collected 1-2 primaries, secondaries, or breast feathers by pulling them from live-captured individuals. Blood samples were typically stored in "Longmire" preservation buffer (Longmire et al. 1988), or less frequently stored in 90% ethanol. Feathers were stored in dry paper envelopes until processed in the laboratory.

Sexing of individual birds

We determined the sex of most individuals using molecular techniques. However, collected individuals were sexed by direct examination of gonads during specimen preparation, and a few live individuals were sexed by the presence of a distended cloaca indicating a female recently laid an egg (Pyle 2008, Jae-Ik et al. 2009). For molecular methods, we used blood and feather samples as a source of DNA to determine sex by PCR amplification of the W and Z chromosomes. From feathers, we used the calamus of sampled primary (~15 mm of the base of a primary feather), secondaries (~10 mm of the bases of two secondary feathers), or up to five breast feathers (~5 mm of the bases). All feather calamus samples were minced into pieces < 1 mm^3. Following 24-hrs incubation with proteinase, feather pieces were no longer visible in solution. We extracted DNA using a "salting out" protocol described in Medrano et al. (1990), modified for feathers by adding 0.5 mg of dithiothrietol during lysis and 0.02 mg of glycogen during precipitation, and for blood and feather samples by adding 0.7 volumes of 2-propanol in place of 2 volumes of ethanol for DNA precipitation. Blood-derived DNA extractions were quantified using fluorometry and diluted to 50ng/µL working solutions; feather-derived DNA was dissolved in 20 µl of water (< 50ng/µL).

We amplified portions of the CHD-W/CHD-Z genes via the PCR using the P2 and P8 primers identified in Griffiths et al. (1998). The P8 primer was synthesized with

an additional modified 19 bp universal tail (LI-COR Inc., Lincoln, NE) added to the 5' end of the oligonucleotide (Steffens et al. 1993, Oetting et al. 1995). A primer with the sequence complementary to the tail, directly labeled with the infrared fluorophore, was used as the fluorescently-labeled primer for the detection of alleles. PCR amplifications were carried out in a final volume of 10 μL and contained ~50 ng genomic DNA, 0.1 μM dNTPs, 0.384 μM P2, 0.4 μM tailed P8, 0.016 μM IRD-labeled primer, 0.2 μg BSA, 1X PCR buffer (Perkin Elmer Cetus I), and 0.5 units Amplitaq DNA polymerase (Promega, Forest City, CA). PCR reactions began with 94° C for 2 min and continued with 40 cycles each of 94°C for 30 sec; 48° C for 45 sec; 72° C for 45 sec, followed by a 1-min annealing at 48° C and a 5-min extension at 72°C to conclude. We electrophoresed fluorescently-labeled PCR products through an 18-cm, 6% polyacrylamide gel on a LI-COR 4200L automated sequencer (LI-COR, Lincoln, NE). We scored images using GeneImageIR™ 4.05 software (Scanalytics, Inc., Fairfax, VA). For quality control purposes, we conducted repeat PCRs for 70 individuals (13%), including 17 repeated blood-derived DNA samples and 54 repeated feather-derived DNA samples. For confirmation of molecular diagnosis of sex we also included samples from three males and five females whose sex had been confirmed by dissection (Fig. 3.3).

Data analysis

To address the issue of a small proportion of missing morphometric measurements, we used a restricted maximum likelihood method (REML) to generate values for missing data, hereafter referred to as "imputed data" (Beale and Little 1975, Krzanowski 1988). This method generated expected values for missing data from the mean and covariance matrix derived from non-missing data. Imputed data were generated independently for each subspecies. Using imputed data allowed us to obtain more robust sample sizes for individual subspecies. This method has been shown to generate reliable estimates for missing measurements in morphometric analyses (Strauss et al. 2003). Having data for all measurements for each subspecies was necessary for subsequent statistical calculations. We imputed total head lengths for 41 individuals (8% of n = 491)

including three male and one female *actites* (16%, n = 25), nine male and four female *kistchinski* (25%, n = 53), and 17 male and seven female *sakhalina* (67%, n = 46).

We used principal components analysis to reduce the morphological measurements into composite scores while maximizing the variance of the characters (Zar 1999). We plotted the first (PC1) and second (PC2) principal components against each other for the four subspecies that co-occur in Asia and the two subspecies that co-occur in Western Alaska to illustrate overlap in morphological measures. All models were created using a set of individuals whose sex was determined via molecular techniques or dissection. The "prior probabilities" for each potential sex or subspecies category were set as equal across groups (e.g. equal chance of being either sex or subspecies). We present the top one or two models according to the highest correct classification rate and highest squared canonical correlation (SCC) values for each model set. We used JMP (8.0.2) for all statistical analyses.

Dunlin are moderately sexual dimorphic; accordingly sex-specific analysis was conducted separately (Browning 1991, Engelmoor and Roselaar 1998). We tested for differences in each morphometric character between the sexes for each subspecies using a one-way analysis of variance (ANOVA) with Tukey-Kramer honestly significant difference (HSD) tests for multiple comparisons. We used morphometric measures to create five independent discriminant function models for determining sex of individuals captured within each subspecies' breeding range. For discriminant function models to identify sex of individual subspecies, we present the two best models to predict sex with and without body mass due to the within season variation in this character. We reported coefficients to determine sex of individuals when subspecies was known that can be used to derive the most likely sex based on the cutting score. Sex classification can be determined by calculating the discriminant function score; values above the model's cutting score were classified as females (Hair et al. 2006).

We constructed discriminant function models to determine subspecies and sex of individual Dunlin during the non-breeding season where various assemblages of Beringia Dunlin subspecies congregate at different stages of their annual cycle and are difficult to

distinguish. Because body mass varies among seasons (e.g. breeding, winter vs. migration), we excluded mass from all non- breeding ground models. The breeding ranges of *pacifica* and *arcticola*'s adjoin in northwestern Alaska and individuals of both subspecies are known to overlap during a post-breeding staging period on the Yukon-Kuskokwim Delta, in Western Alaska (Warnock and Gill 1996). We reported coefficients to determine subspecies (*arcticola* and *pacifica*) when the sexes of individuals were known and unknown; these values can be used to derive the most likely subspecies based on the cutting score. Subspecies classification can be determined by calculating the discriminant function score; values above the model's cutting score were classified as *pacifica*.

Four subspecies comingle during the non-breeding season along the East Asian-Australasian Flyway including *arcticola*, *actites*, *kistchinski*, and *sakhalina* (Lanctot et al. 2009). We developed models for these two staging/wintering areas under two scenarios where an individual's sex was known, but not its subspecies status, and where an individual's sex was unknown but not its subspecies status. This allows workers to determine sex and subspecies ratios for mixed subspecies assemblages. Subspecies classification can be determined by calculating each of the four subspecies- specific function scores separately. The most probable subspecies yields the highest discriminant function score (Hair et al. 2006). We reported constants and coefficients to determine subspecies (*actites*, *arcticola*, *sakhalina*, *kistchinski*) when the sex of individuals were known, that can be used to derive the most likely subspecies based on the discriminant function model.

RESULTS

DNA markers of sex

CHD amplification products separated as three distinctly sized fragments (Fig. 3.3). Birds of known sex collectively exhibited two Z alleles (363 bp and 368 bp) and one W allele (394 bp). Males (ZZ: 363 bp and/or 368 bp) were distinguishable from females (WZ: 394 bp plus 363 bp or 368 bp) based on the presence of the W allele. We used

DNA markers to determine the sex of 542 individuals. Identical results were obtained from 58 separate PCRs of the same blood-derived DNA samples and 68 separate PCRs of the same feather-derived DNA samples. Separate PCR amplifications of independently collected tissue samples from the same individual in different years gave identical results in 79 of 80 cases. The one contradiction was an individual in which a blood sample and a feather sample each amplified as male, while a second feather sample collected in another year amplified as female (5 times). We could not distinguish between misidentification, mislabeling, contamination, or mutation as the cause for this difference, and consequently did not use it in developing discriminant function models. Nevertheless, our error rate (1.25%) was very low and subsequently we felt confident that our DNA sex identification was sufficiently accurate to develop discriminant models.

Morphometric comparisons between subspecies and sex

Among the five subspecies of Dunlin examined, *actites* was the smallest and *pacifica* the largest for all morphological measures (Fig. 3.4). Females were larger on average than males across each subspecies and most morphometric characters. We found significant differences between the sexes for all morphometric characters of Dunlin except body mass for the *pacifica* subspecies (Fig 3.4).

The first principal component (PC1) accounted for 67.1% and 62.0% of the variance in morphometrics for females and males, respectively, with all subspecies pooled (Table 3.1). The second principal (PC2) accounted for 14.7% and 15.7% of the variance for females and males, respectively. Cumulatively, PC1 and PC2 accounted for a total of 81.8% of female variance and 77.7% of male variance. PC1 is likely a composite index of size, while the negative scores for PC2 indicate it is likely a composite index of shape. We plotted individual subspecies PC1/PC2 scores by sex for subspecies that overlap during migration or on the wintering ground: including *actites*, *arcticola*, *sakhalina* and *kistchinski* (Fig. 3.5) and *pacifica* and *arcticola* (Fig. 3.6). Considerable overlap exists among subspecies, with the exception of *actites* where both males and females had consistently lower PC1 scores than the other subspecies indicating

they were smaller (Fig. 3.5). The *arcticola* subspecies tended to have lower PC1scores than the *pacifica* subspecies suggesting it was generally smaller than the *pacifica* subspecies (Fig. 3.6).

Discriminant function models were able to predict the sex of individuals within each subspecies with reasonably high accuracy, >83% in all cases, but generally above 90% (Table 3.2). Individuals belonging to the *kistchinski* subspecies could be separated into males and females with the highest classification accuracy, while the *arcticola* subspecies had the poorest classification accuracy. Models to determine sex of *pacifica* and *arcticola* staging in Western Alaska and *actites*, *arcticola*, *kistchinski* and *sakhalina* wintering along the Australasian Flyway had lower (75-87%) classification accuracies (Table 3.3). In general, models with more variables had higher classification rates.

The best discriminant function model for distinguishing between the two subspecies of Dunlin staging in western Alaska (*arcticola* and *pacifica*), when sex was unknown, correctly classified *pacifica* and *arcticola* 78% and 69% of the time, respectively, for a total classification rate of 73% (Table 3.4). Discriminant function model could more accurately classify *arcticola* and *pacifica* when sex was known (Table 3.3). For known sex birds, the best model for males correctly classified individuals > 80% of the time and the best model for females correctly classified individuals 75- 81% of the time.

A discriminant function model to separate the four subspecies that winter together in Asia, *actites*, *arcticola*, *kistchinski*, and *sakhalina,* had a relatively low overall classification rate of 60% for all groups when sex was unknown, with *actites* having the highest classification of 96%, *kistchinski* at 72%, and *arcticola* and *sakhalina* below 45%. We did not report a classification model function for the Asian wintering subspecies assemblage (listed above) due to the low correct classification rates across groups. Models for known male and females had higher overall classification accuracies (Table 3.5). The best model for males was highly accurate for *actites* (100%), but less accurate for the other subspecies (< 60%). The best model for female was also accurate for *actites* (100%) and *kistchinski* (94%), but less accurate *arcticola* and *sakhalina*.

DISCUSSION

We examined the feasibility of using morphometric characters alone to classify subspecies and sex of Beringia Dunlin using principal component and discriminant function analyses. Beringia Dunlin have diverse migration strategies and population-specific limiting factors that vary by sex (e.g. survival, staging locations, migration routes, wintering locations). Therefore, it is important to be able to reliably identify sex of individuals to effectively understand the sex-specific factors that limit population growth. While molecular techniques yield higher rates of accuracy of sex determination, discriminant function models provide a cost effective and reliable alternative for determining sex. Our discriminant function models separated all five subspecies into males and females with high classification accuracies (87-98%). Other studies attempting to determine sex of waterbirds using discriminant function analysis report similar ranges (76-96%) of classification accuracy (Brennan et al. 1984, Jodice et al. 2000, Gunnarsson et al. 2006, Shealer and Cleary 2007, Meissner and Pilacka 2008). Studies incorporating sex-specific estimates of survival, site fidelity, sex ratio of offspring, lifetime productivity, and natural history are greatly enhanced when sex can be determined. Conversely, analyses without sex-specific data can lead to erroneous interpretation (Hanowski and Niemi 1990, Dimmick and Pelton 1994, Ellegren and Sheldon 1997, Nichols et al. 2004). Our models for determining sex for subspecies groups (e.g. mixed groups of *arcticola* and *pacifica*, or mixed groups of *actites*, *arcticola*, *kistchinski* and *sakhalina*) were accurate 79-85% accurate in determining sex of Beringia Dunlin and can be used to determine sex at during different periods of their life cycle. The equations we derived from discriminant function models can be useful for researchers for determining sex ratios of migratory and wintering populations where known subspecies intermix.

The ability to differentiate subspecies is also important for deriving subspecies-specific population estimates and can be used to determine subspecies identity in mixed flocks when subspecies aggregate on the migrating and wintering grounds (i.e. proportion of subspecies/population). Our discriminant function models were able to separate

individuals into subspecies with variable accuracies with accuracy improving when sex was first determined. Researchers using similar analytical tools to discriminate subspecies of Canada Goose (*Branta canadensis*) and Arctic Warbler (*Phylloscopus borealis*) reported higher rates of correct classification of 76 – 96% (Merendino et al. 1994, Saitoh et al. 2008). Poor classification accuracies for Beringia Dunlin species are likely due to real overlap in morphometric measures, as well as the result of having small samples sizes for the Russian-breeding subspecies. We found that *arcticola* and *sakhalina* Dunlin had the lowest correct classification rates and most overlap in morphological characteristics. Subspecies with overlapping breeding ranges had lower classification accuracies; especially *arcticola*, *sakhalina* and *kistchinski* subspecies. Although some uncertainty in classification still exists, our models provide an inexpensive alternative to more costly techniques (e.g. stable isotopes and molecular techniques) for classifying Dunlin (Lanctot et al. 2009).

Subspecies designations for Dunlin previously relied on a combination of differences in morphometric measures and variation in alternate plumage, molt schedules, and migration routes. Thus it is not surprising that morphometrics alone had limited accuracy in classifying subspecies in some cases. Browning (1991), when examining specimens collected on the breeding grounds, noted differences in alternate plumage color and pattern between *arcticola*, *sakhalina*, and *kistchinski* and found high overlap in morphometrics of each subspecies. Flight feather molt schedules can also be useful in distinguishing Dunlin subspecies during post-breeding staging in Western Alaska. Both *sakhalina* and *arcticola* molt their innermost primaries during incubation while *actites*, *kistchinski*, and *pacifica* do not initiate flight feather molt until after incubation (Holmes 1971, Tomkovich 1998). Depending on timing of capture, individuals with new flight feathers during fall migration can be separated using flight feathers alone.

Management and conservation of long-distance migratory bird populations often requires a detailed understanding of migratory connectivity in addition to productivity and survival estimates. Insufficient information on the distribution of subspecies within the East Asian Australasian flyway (EAAF) hinders effective conservation and

management of shorebirds. In order to gain the most from our subspecies results, we recommend researchers collect a blood sample to determine sex of individuals. After sex has been determined, using the mixed subspecies discriminant function model equation for the appropriate group will yield a reliable classification of the Dunlin subspecies.

Finally, approximately 70% of the global populations of *actites*, *arcticola*, *kistchinski*, and *sakhalina* utilize the Yellow Sea during northward migration (Barter 2002). Among the subspecies wintering in Asia, *actites* is the smallest, most distinctive subspecies; it is also of greatest conservation concern because of its small population size (<900 individuals, Bamford et al. 2008). Current development activities including coastal reclamation of the Yellow Sea have the potential to negatively impact Dunlin populations in the EAAF. Our ability to reliably classify *actites* within mixed subspecies groups will aid researchers in identifying wintering and migration stopover sites for this subspecies that can lead to conservation of important habitat for sustaining Dunlin populations.

ACKNOWLEDGMENTS

This project represents an international effort to understand the geographic distribution of staging and wintering Beringia Dunlin and develop tools for differentiating them during field studies. Samples were collected by numerous individuals and workers were supported by many institutions, agencies and foundations. We thank C. Dau for providing tissue samples for known sex individuals. D. Tracy for collecting *pacifica* samples; S. Sotnikov and O. Valchuk for collecting *actites* samples, T. Miller and S. Drovetski, for collecting *kistchinski* samples; P. Tomkovich for collecting *sakhalina* and *kistchinski* samples. K. Sage and S. Talbot from the U.S. Geological Survey Alaska Science Center's Molecular Ecology Laboratory processed molecular samples for *arcticola* and *actites.* K. Broadway, C. Chiang, J. Choi, N. Coutsoubos, B. Hill, A. Taylor, and G. Thomas collected *arcticola* samples. We thank J. Johnson for map preparation. Funding and logistical support was provided by U. S. Bureau of Land Management, Arctic Field Office; U.S. Fish and Wildlife Service's Migratory Bird Management Program; U.S. Geological Survey, Alaska Cooperative Fish and Wildlife Research; University of Alaska Fairbanks; and Arctic Audubon. The use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the US Government.

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Table 3.1. First and second principal component scores (PC1 and PC2), eigenvalues, and the percent of variance explained for five morphometric measures for five subspecies of female and male Dunlin (*Calidris alpina actites*, *arcticola*, *kistchinski*, *pacifica* and *sakhalina*).

	Female		Male	
	PC1	PC2	PC1	PC2
Culmen	0.48	0.11	0.52	0.06
Total Head	0.51	-0.03	0.52	0.10
Tarsus	0.35	0.81	0.35	0.72
Wing	0.46	-0.21	0.38	-0.65
Mass	0.41	-0.53	0.44	-0.20
Eigenvalue	3.36	0.74	3.10	0.78
Variance %	67.1	14.7	62.0	15.7
Cumulative %	67.1	81.8	62.0	77.7

Table 3.2. Discriminant function models for determining the sex of adult Dunlin (*Calidris alpina*). Models were developed from five morphometric measures: exposed culmen (EC), total head (TH), diagonal tarsus (DT), flat wing (FW), and body mass (BM). Models were developed separately for the five subspecies: *C. a. actites*, *kistchinski*, *sakhalina*, *arcticola*, *pacifica.* Two models are presented for each subspecies, the first includes mass and the second does not include mass.

Discriminant function models	SCC	Wilks' Lambda	F-value	df	Cutting score >= ♀	Correct classification percent ♀ *n*	♂ *n*	Total *n*
Sex of *actites*						*11*	*14*	*25*
0.6641 * (EC) + 0.1472 * (TH) + 0.8976 * (DT) - 0.0125 *(FW) - 0.1271 *	0.790	0.375	6.32	19	43.74	92	100	96
0.6747 * (EC) + 0.7659 * (DT)	0.771	0.405	16.11	22	39.68	83	100	92
Sex of *kistchinski*						*17*	*26*	*43*
0.5955 * (EC) + 0.1684 * (BM)	0.888	0.211	74.63	40	30.50	100	96	98
0.5549 * (EC) + 0.0384 * (TH) + 0.2869 * (DT) + 0.1395 * (FW)	0.881	0.224	32.83	38	47.18	100	96	98
Sex of *sakhalina*						*10*	*26*	*36*
0.4531 * (EC) + 0.6021 * (TH) + 0.5504 * (DT) + 0.1094 * (FW) + 0.1474 *	0.915	0.162	30.94	30	56.14	90	100	97
0.3951 * (EC) + 0.2703 * (FW)	0.774	0.400	24.73	33	47.31	100	96	97
Sex of *arcticola*						*181*	*160*	*341*
0.4513 * (EC) + 0.0615 * (DT) + 0.1244 * (FW) + 0.0762 (BM)	0.734	0.460	98.2	336	37.31	88	86	87
0.5049 * (EC) + 0.1034 * (DT) + 0.1408 * (FW)	0.721	0.480	121.5	337	37.87	87	85	86
Sex of *pacifica*						*20*	*26*	*46*
0.2743 * (EC) + 0.2794 * (TH) + 0.2227 * (DT) + 0.1468 * (FW) + 0.0102 *	0.861	0.257	23.0	40	52.70	95	96	96
0.2855 * (EC) + 0.2693 * (TH) + 0.2215 * (DT) + 0.1489 * (FW)	0.861	0.258	29.4	41	52.12	95	96	96

Table 3.3. Discriminant function model for determining sex of adult Dunlin (*Calidris alpina*). Models include 1) four subspecies known to congregate along the Australasian Flyway (e.g. *actites*, *kistchinski*, *sakhalina*, or *arcticola*); and 2) two subspecies known to congregate in western Alaska (*e.g. arcticola* and *pacifica*). Models were developed from four morphometric measures: exposed culmen (EC), total head (TH), diagonal tarsus (DT), and flat wing (FW).

						Correct classification percent		
		Wilks'			Cutting score	♀	♂	Total
Discriminant function models	SCC	Lambda	F- value	df	>= ♀	*n*	*n*	*n*
Sex of *actites*, *kistchinski*, *sakhalina*, and *arcticola*						*60*	*84*	*144*
0.0521 * (EC) + 0.3196 * (TH) - 0.1036 * (DT) - 0.0204 * (FW)	0.559	0.687	15.78	139	15.30	75	82	79
Sex of *arcticola* and *pacifica*						*46*	*54*	*100*
0.1324* (EC) + 0.2499* (TH) + 0.1646 * (FW)	0.718	0.484	34.07	96	40.46	83	87	85

Table 3.4 Discriminant function models to determine subspecies of adult Dunlin (*Calidris alpina*) as either belonging to the *arcticola (arc)* or *pacifica (pac)* subspecies when sex is and is not known. Models were developed separately for males and females, and males and females combined (to stimulate sex unknown) from combinations of four morphometric measures: exposed culmen (EC), total head (TH), diagonal tarsus (DT), and flat wing (FW).

						Correct classification percent		
Discriminant function model	SCC	Wilks' Lambda	F-value	df	Cutting score > = *pac*	*arc* n	*pac* n	Total n
Subspecies of *arcticola and pacifica* when sex is unknown						*54*	*46*	*100*
0.6755 * (EC) - 0.2210 * (TH) + 0.0152 * (DT) - 0.1578 * (FW)	0.553	0.693	10.4	95	-8.33	69	78	73
Subspecies of *arcticola and pacifica* when sex is male						*28*	*26*	*54*
0.7476 * (EC) - 0.0833 * (TH) + 0.0745 * (DT) - 0.0617 * (FW)	0.653	0.573	9.1	49	15.14	82	89	85
0.7537 * (EC) - 0.0937 * (TH) - 0.0663 * (FW)	0.652	0.575	12.3	50	12.21	82	89	85
Subspecies of *arcticola and pacifica* when sex is female						*26*	*20*	*46*
0.6299 * (EC) - 0.0203 * (TH) + 0.0532 * (DT) - 0.0432 *(FW)	0.726	0.473	11.4	41	18.73	81	75	78
0.6104 * (EC) + 0.0521 *(DT) - 0.0440 * (FW)	0.726	0.473	15.6	42	19.15	81	75	78

Table 3.5. Discriminant function models to identify subspecies of adult Dunlin (*Calidris alpina*) whose sex is known as belonging to the *actites* (*act*), *arcticola (arc)*, *kistchinski* (*kis*) or *sakhalina* (*sak*) subspecies. Models were developed separately for male and females from four morphometric measures: exposed culmen, diagonal tarsus, total head, and flat wing.

				Correct classification percent				
Discriminant function models	SCC	Wilks' Lambda	F-Est.	*act* *n*	*arc* *n*	*kis* *n*	*sak* *n*	Total *n*
Subspecies of *actites, arcticola, kistchinski*, *sakhalina* when sex is male				*13*	*19*	*26*	*26*	*84*
	0.881	0.194	20.3	100	58	39	53	57
Subspecies of *actites, arcticola, kistchinski*, *sakhalina* when sex is female				*12*	*21*	*17*	*10*	*60*
	0.867	0.151	17.1	100	57	94	60	77

Table 3.6. Discriminant function model constant and coefficients that can be used to classify an individual adult Dunlin (*Calidris alpina*) as either belonging to the *actites*, *arcticola*, *kistchinski* or *sakhalina* subspecies when sex is known. Models were developed from morphometric measures: exposed culmen, diagonal tarsus, total head, and flat wing. n/a indicates the character was not included in the model.

	Constant	Culmen	Total head	Tarsus	Wing
Male					
actites	-5447.489	-157.970	197.429	n/a	45.032
arcticola	-6265.053	-167.900	211.802	n/a	47.863
sakhalina	-6137.366	-164.803	209.203	n/a	47.199
kistchinski	-6102.507	-164.547	208.142	n/a	47.346
Female					
actites	-2390.473	17.0620	n/a	5.384	35.141
arcticola	-2730.033	19.5194	n/a	4.663	37.429
sakhalina	-2777.058	21.4103	n/a	6.340	36.876
kistchinski	-2821.453	19.8164	n/a	4.965	38.010

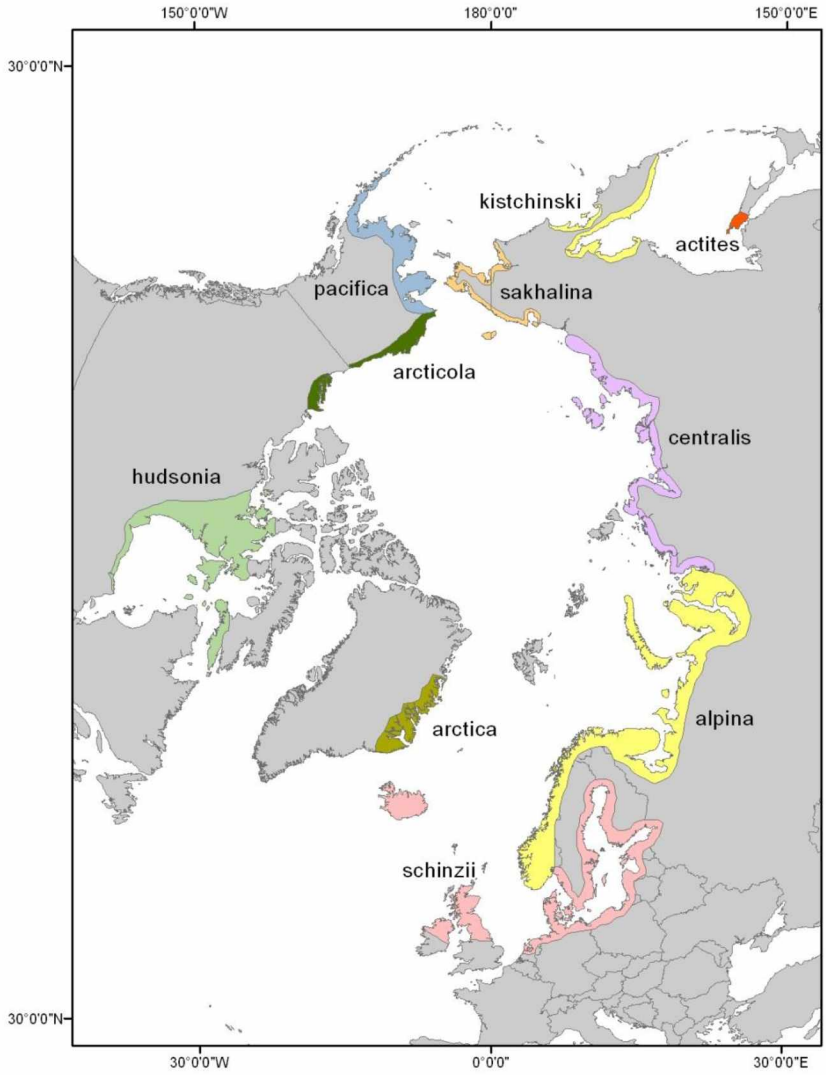

Figure 3.1 Holarctic breeding distribution of Dunlin (*Calidris alpina*), including ranges of 10 subspecies according to morphometric variation (Engelmoor and Roselaar 1998). Subspecies breeding ranges were based on original taxonomic descriptions and current knowledge (Tomkovich 1986, Browning 1991, Tomkovich and Zharikov 1997, Engelmoor and Roselaar 1998, Tomkovich and Serra 1999).

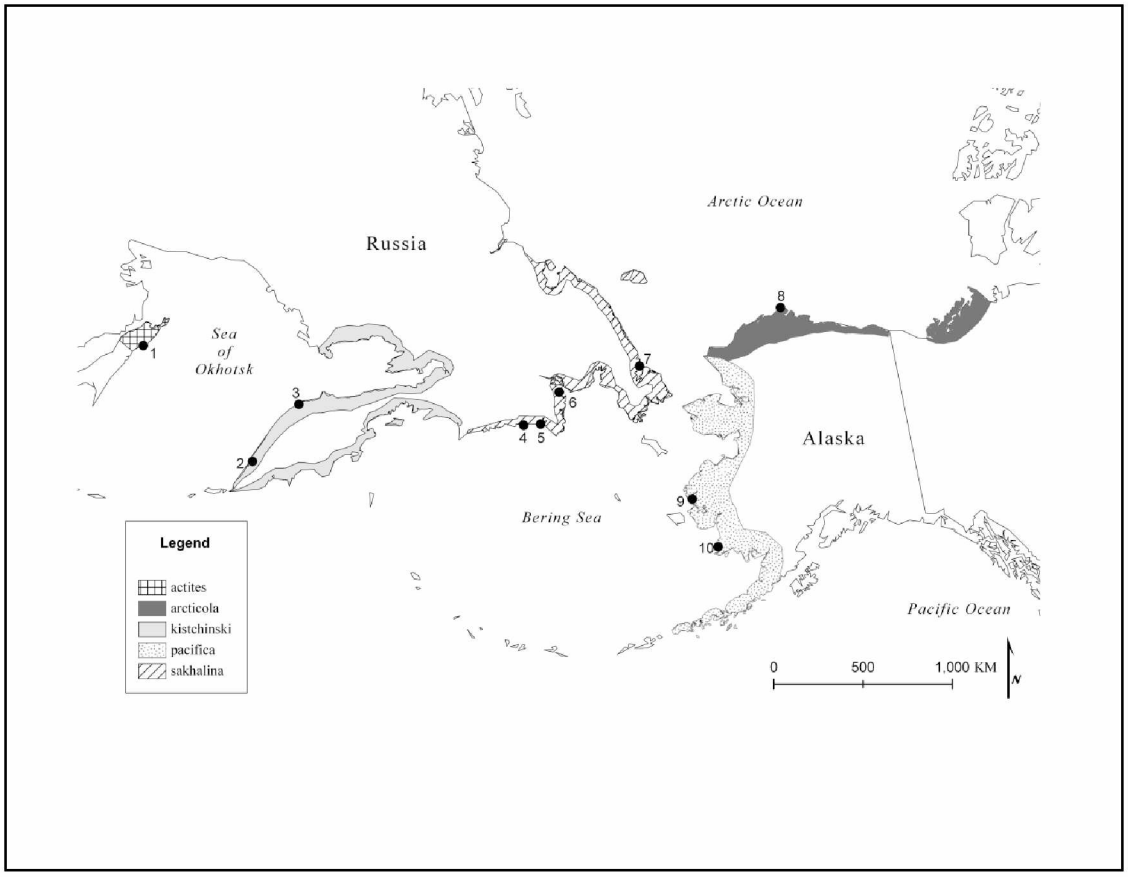

Figure 3.2 Breeding distribution and sampling sites (n = 10) for the five Dunlin (*Calidris alpina*) subspecies included in this study. Subspecies range designations were based on current knowledge of breeding ranges according to original taxonomic descriptions and field studies (Tomkovich 1986, Browning 1991, Tomkovich and Zharikov 1997, Tomkovich and Serra 1999, Engelmoor and Roselaar 1998). Sampling sites are: Site 1 = Chaivo Bay, Site 2 = Southwest, Site 3 = Fchun, Site 4 = Khatyr, Site 5 = Meinypilgno, Site 6 = Vtoraya, Site 7 = Belayaka, Site 8 = Point Barrow, Site 9 = Manokinak, Site 10= Platinum Spit. See text for details.

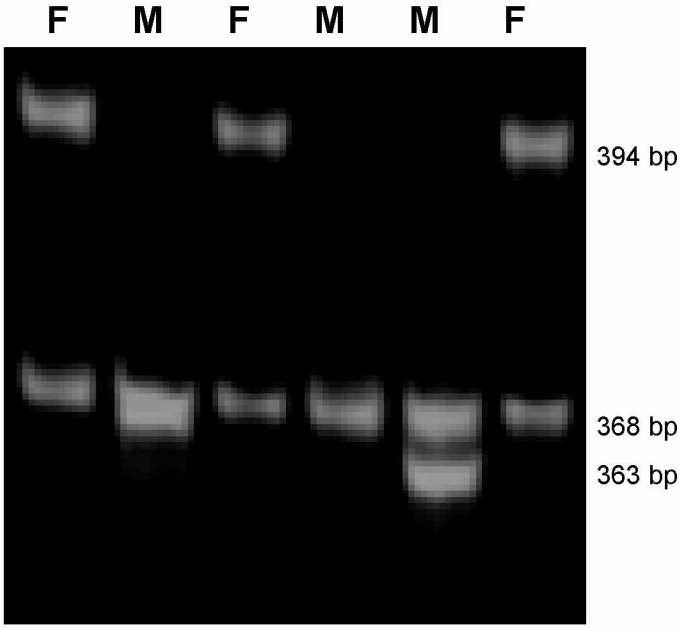

Figure 3.3 Fragment sizes from amplification of CHD using P2/P8 primers in Dunlin (*Calidris alpina*). Sexes were confirmed by gonadal examination and are identified at the top of the panel.

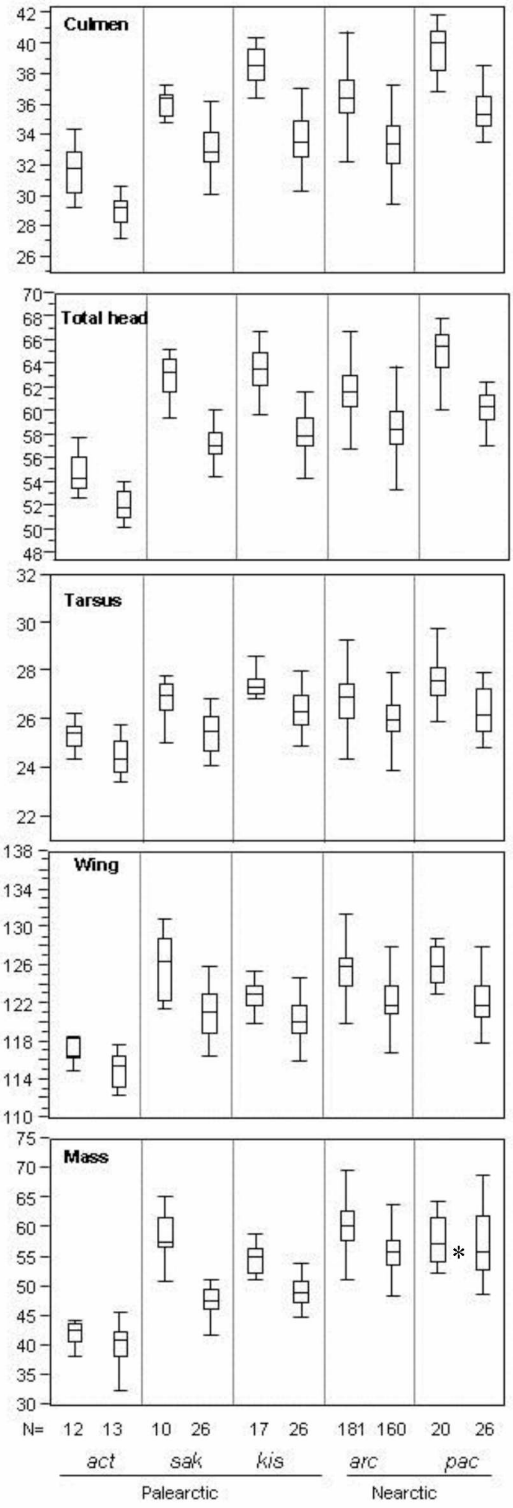

Figure 3.4. Median (horizontal bar within box), lower quartile (lower edge of box), upper quartile (upper edge of box) and minimum (lower bar) and maximum (upper bar) values for male and female Dunlin (*Calidris alpina*) exposed culmen (mm), total head (mm), diagonal tarsus (mm), flat wing (mm), and body mass (g) measures. Female values are plotted to the left of males. Numbers in parentheses below the x-axis labels are samples sizes Subspecies along the x-axis are organized from smallest to largest: *C. alpina actites* (act), *sakhalina* (sak), *kistchinski* (kis), *arcticola* (arc), and *pacifica* (pac). * indicates no significant difference ($p > 0.005$) between the sexes of a given subspecies and morphometric measure.

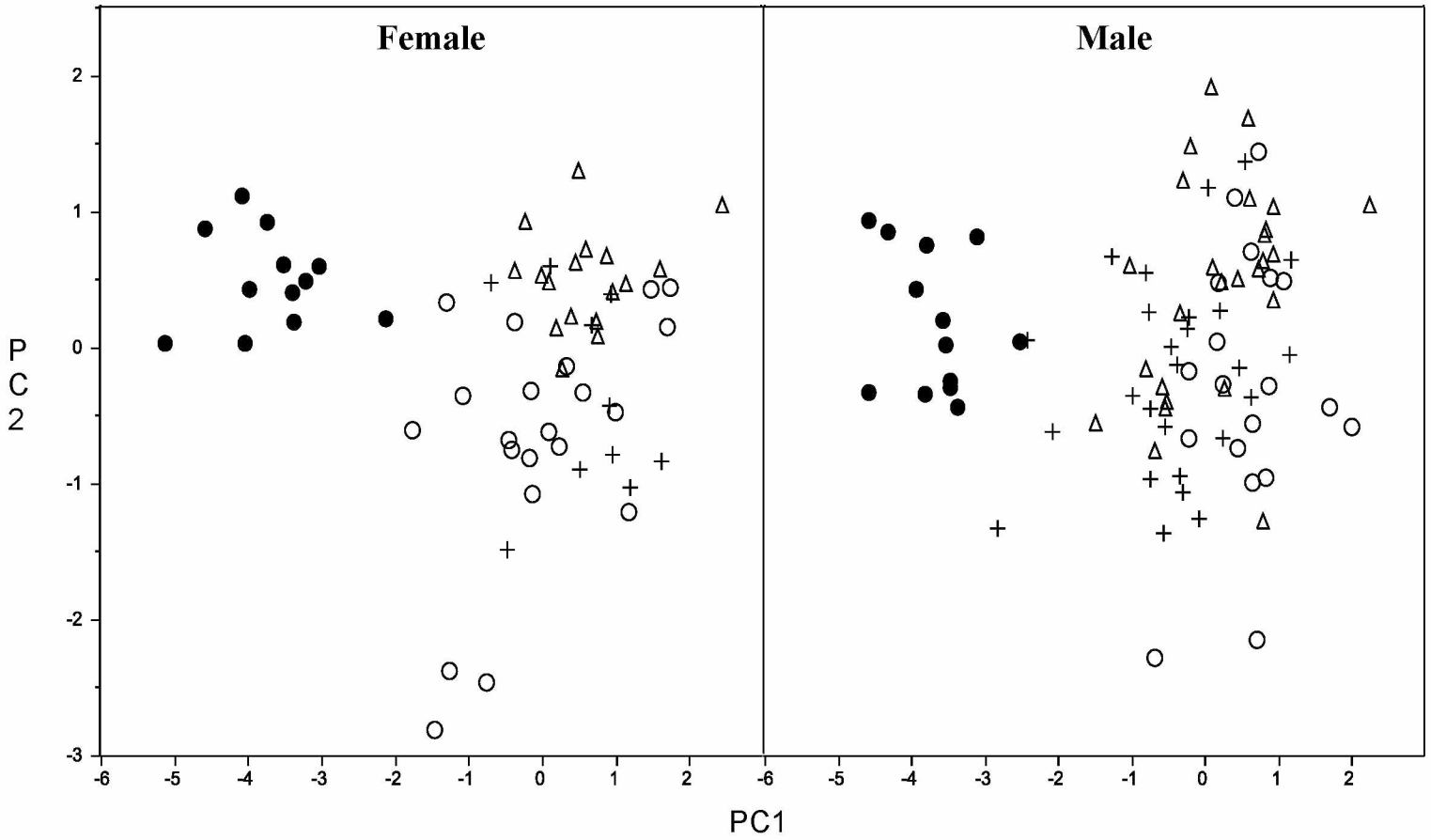

Figure 3.5 Principal component scores 1 and 2 derived from five morphometric measures for male and female Dunlin (*Calidris alpina*) subspecies: *actites* (solid circles), *arcticola* (open circles), *kistchinski* (open triangles) and *sakhalina* (+).

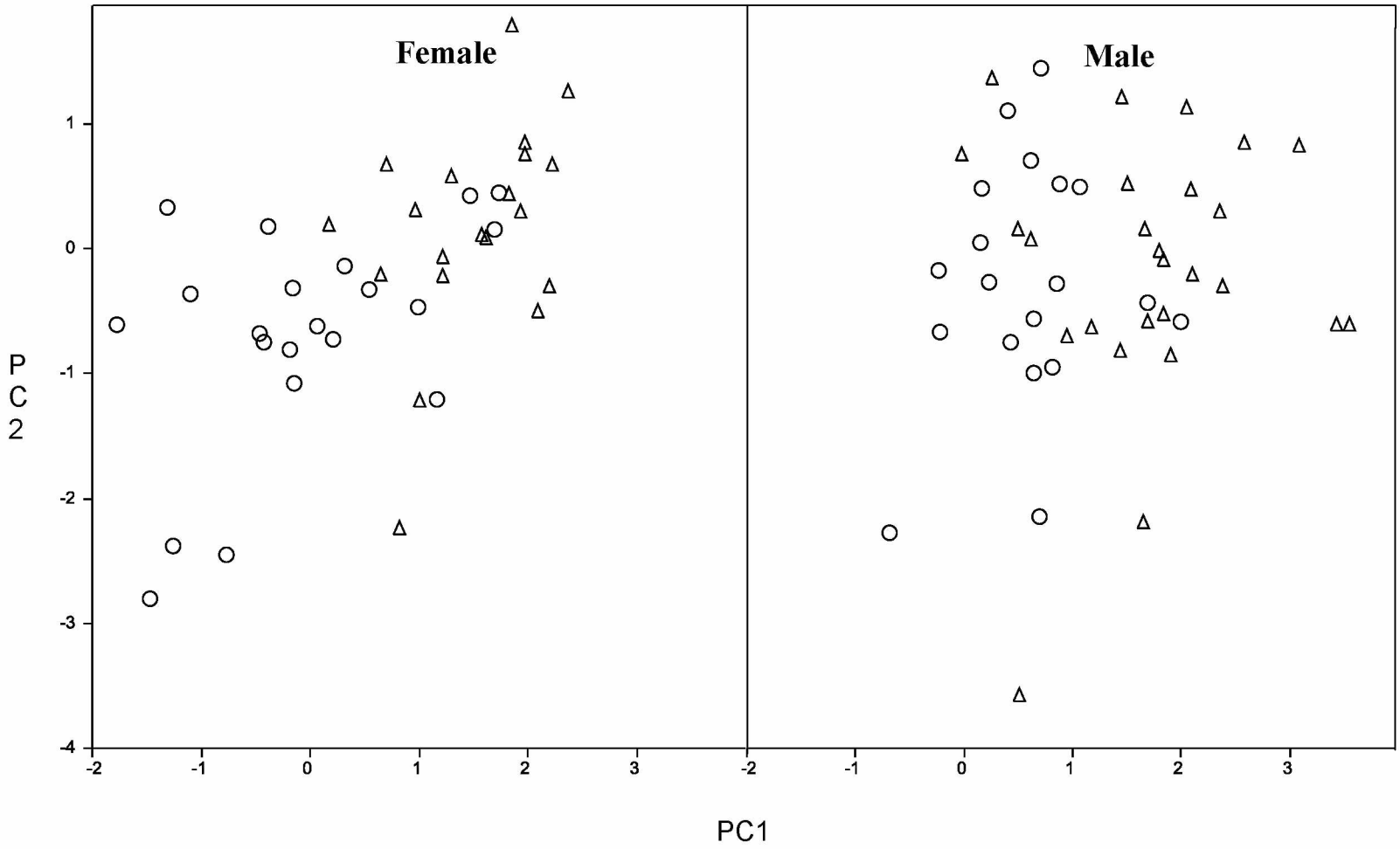

Figure 3.6. Principal component scores 1 and 2 derived from five morphometric measures for male and female Dunlin (*Calidris alpina*) subspecies: *arcticola* (open circles) and *pacifica* (open triangles).

CHAPTER 4: CONCLUSIONS

I studied an important component of fecundity for Arctic-breeding shorebirds by documenting high rates of renesting (e.g. replacement clutch laying) in Dunlin. By experimentally removing clutches either early or late in incubation, I tested whether Dunlin were more or less likely to renest depending on incubation stage. I found that clutches lost early in incubation were mostly (85-95%) replaced and documented a lower renesting rate (35-50%) as incubation progressed. Such high rates of clutch replacement laying by an Arctic-breeding bird were surprising given the temporal constraints and energetic demands of long-distance migration in addition to an abbreviated nesting period (Tulp 2007, Soikkeli 1967). Documentation of replacement clutches requires following a uniquely marked individual after clutch loss. Only a few studies working with waterfowl have addressed this question using experimental clutch removal paired with the use of radio transmitters to follow individuals (Arnold et al. 2010, Flint et al. 2006, Grand and Flint 1996). No studies have used experimental techniques to study clutch replacement in shorebirds. Documenting renesting is further confounded when predation rates are high early in the season, when most individuals are not uniquely marked, or when divorce occurs and individuals move beyond the search area to renest with new mates. Other studies examining renesting ecology for Arctic-breeding shorebirds have documented replacement clutch laying in a natural setting and are likely biased low due to difficulties studying renesting ecology (Naves et al. 2008).

The high rates of replacement clutch laying and the relatively close geographic distance between initial and replacement nests suggests that current estimates of Arctic-breeding shorebird densities (and population estimates) obtained from the Program for Regional and International Shorebird Program (PRISM, Bart and Earnst 2002, Bart et al. 2005) are biased high. Basic methods employed for PRISM surveys include three visits to locate all shorebird nests in a 400 X 400 meter intensive plot. Because birds are not marked during PRISM surveys, it is likely that nests found later in the season could be

the replacement clutches rather than initial nests. Thus, the numbers of breeding birds would be overestimated because individuals could lay both an initial and replacement clutch within a single plot. This may be particularly problematic in years where predation rates are high and birds are likely to lay replacement clutches.

The results warrant further investigation into the phenomenon of replacement clutch laying in other Arctic-breeding shorebird species. If renesting rates remain high like I observed in Dunlin, then replacement clutches should be taken into account (perhaps by removing nests initiated later in the season) in density estimation procedures. Not doing so could bias and inflate population estimates of species. Having an incomplete understanding of a vital demographic parameter, such as replacement clutch rates, hinders managers' ability to generate reliable population estimates to effectively manage populations.

My study was focused on evaluating whether replacement nests were laid frequently by Dunlin and identifying factors that contributed to clutch replacement (e.g. timing of nest loss and body condition of adults). Further investigation of the importance of age class and experience may influence an individual's propensity to lay second or even third clutches within a year. It would also be worthwhile to compare the quality (e.g. mass at hatch, growth rates of chicks during the brood rearing period) and survival of offspring from replacement clutches. Finally, other factors that might be considered include environmental variables (e.g. predator numbers, food availability, seasonal weather patterns) that may affect propensity to replace clutches.

Chapter 3 examined the reliably of distinguishing Beringia Dunlin subspecies using solely morphometric measures, which are easily obtained in the field. My discriminant function models were able to differentiate subspecies that overlap during migration or the wintering period with variable correct classification rates. The models to separate staging *pacifica* and *arcticola* Dunlin had 78-85% classification accuracy when sex was known and these rates declined to 73% when sex was unknown. Results from these analyses can be used by researchers who are working to identify the staging sites used by Dunlin populations in Alaska. The models to distinguish four subspecies (*actites*,

arcticola, kistchinski and *sakhalina*) of Dunlin wintering and migrating through the East Asia Australasian Flyway were less accurate but were highly successful in correctly predicting the *actites* subspecies, which has a small population size. Results from these analyses allow researchers to identify relative proportion of Dunlin subspecies at wintering and migration locations. This in turn can be used to understand the importance of stopover sites and wintering locations by different subspecies. A greater understanding of mixed subspecies composition during migration or winter will assist in efforts to understand the decline of particular subspecies, especially those with very small population sizes (e.g. *actites*) that may be disproportionately impacted by human development on the wintering grounds.

All of my discriminant function models improved when the sex of individuals was known prior to determining subspecies. Therefore, I recommend that researchers extract a blood or feather sample to determine molecular sex before using the discriminant function models equations to classify subspecies. The models to determine sex of five subspecies of Dunlin breeding in Beringia had high correct classification accuracies (87-96%). These models are useful for researchers who wish to determine sex of individuals where subspecies is known either during the breeding or wintering period.

In conclusion, my studies have contributed to the knowledge of Arctic-breeding shorebirds by revealing that renesting is a prevalent strategy employed by a monogamous shorebird to compensate for clutch loss. My study generated renesting rates that can be used in demographic analyses for Dunlin and have encouraged further investigations into renesting ecology in Arctic-breeding shorebirds. Secondly, my investigation into developing discriminant function models to identify Dunlin subspecies and sex via morphometric measures alone has yielded useful tools to assist researchers interested in including the importance of subspecies and sex for breeding and migration ecology of Dunlin. My contributions will result in more precise estimates of Dunlin productivity and greater understanding of the renesting ecology at high latitudes. Additionally, the ability to differentiate Dunlin subspecies and sex will enable researchers to include these factors in future studies of their breeding and migration ecology.

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